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THE NATURAL HISTORY OF ATHEROSCLEROSIS

THE EARLY AORTIC LESIONS AS SEEN IN NEW ORLEANS IN THE MIDDLE OF THE 20TH CENTURY*

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The real significance of atherosclerosis is the role that it plays in the production of human disease. Manipulations of diet, physical activity, or hormonal levels may alter some of the constituents of the blood, and a study of these relationships may yield useful basic information. However, the fact remains that the ultimate criterion for the effectiveness of any proposed prophylactic or therapeutic procedure must be its effect on the clinical manifestations of disease. In the variety of disorders related to atherosclerosis, the universally recognized common denominator is the characteristic arterial lesion, a structural alteration in the arterial wall which obstructs the lumen and reduces blood flow to a part of the body, thereby producing overt clinical manifestations.

Our present concept of the sequence of events in human atherosclerosis leading from lesions to clinical phenomena is diagramatically presented in Text-figure 1. The simple fatty streak is considered to represent the earliest lesion of atherosclerosis that can be recognized with facility either grossly or histologically. The fatty streak is gradually converted into a fibrous plaque, at the base of which a core of lipid usually remains. A fibrous plaque may become sufficiently large to cause slowly progressive stenosis of the lumen of a vessel, particularly if another plaque is located opposite to it. It may undergo sufficient enlargement by accretion of additional lipid on the surface so as to produce a similar effect; or it may become vascularized and

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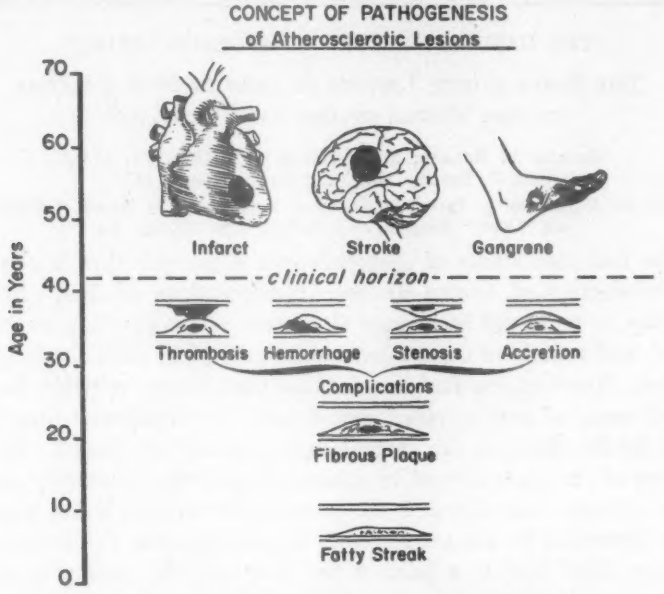
undergo hemorrhage, or ulcerated and be covered by a thrombus. In the latter instances rapid occlusion of the artery may result. Rarely, the lesion may so weaken the underlying media that an aneurysm is produced; or it may become calcified, a change probably representing a healing process.



Text-figure 1. Concept of pathogenesis of atherosclerotic lesions.

The whole gamut of these changes, and particularly those which lead to clinical recognition, have not yet been reproduced in experimental animals. Furthermore, the applicability to man of those experimental vascular lesions which have been produced in animals is not clearly established. This difficulty in correlating the lesions in experimental animals and those in man has prompted us to restudy the natural history of human atherosclerosis in the light of the concept of pathogenesis outlined above.

For this study, the aorta was selected as that part of the vascular system to be preserved and examined. The first step, previously reported in detail,[1] was the grading of 300 aortas procured from consecutive necropsies on individuals of all ages. The observations most significant with respect to the study to be reported here were that simple fatty streaks were found in children as young as 9 months of

age; that every case beyond the age of 7 years had at least minimal fatty streaks; and that only after the age of 30 years did fibrous plaques appear in appreciable numbers of cases. This early study emphasized the need for a more quantitative method of expressing the degree of severity of atherosclerotic lesions, and also demonstrated the desirability of focusing attention on the earlier age groups for more intensive consideration.

This report is based on the study of 461 essentially complete aortas (Table I) and 65 incomplete specimens from 526 patients between 1 and 40 years of age. These patients were examined at necropsy in a large general hospital and medico-legal pathology service in New Orleans over a 5-year period. The more refined methods of processing and grading atherosclerotic lesions have brought to light certain facts about their natural history that suggest important leads for future research.

TABLE I

Tabulation of 461 Cases by Age, Sex and Race

	White			Negro			Total
Age in years	M	F	Total	M	F	Total	
1–5	18	11	29	54	43	97	126
6–10	12	3	15	15	11	26	41
11–15	8	5	13	14	9	23	36
16–20	11	6	17	20	8	28	45
21–25	19	5	24	14	12	26	50
26–30	12	9	21	13	22	35	56
31–35	8	8	16	14	15	29	45
36–40	14	13	27	18	17	35	62
Total	102	60	162	162	137	299	461

MATERIALS AND METHODS

The material on which this report is based was obtained from necropsies performed on individuals between the ages of 1 and 40 years in Charity Hospital of Louisiana at New Orleans, and in the pathology laboratory of the Office of the Coroner, Parish of Orleans, in the 5-year period from 1952 to 1957. Charity Hospital is a 3,076 bed general hospital located in a metropolitan area of approximately 700,000 people, serving patients from the entire state of Louisiana. Patients suffering from all types of both acute and chronic diseases are admitted. As a general rule these patients represent the lower income groups. During the first 4 years of this study, between July 1,

1952, and June 30, 1956, Charity Hospital admitted 276,932 persons, of whom 39,776 were white males; 43,241 were white females; 67,568 were Negro males; and 126,347 were Negro females. Thus the admissions were approximately 30 per cent white and 70 per cent Negro. In this same period, there were 11,173 deaths in this hospital. Necropsies were performed on 5,973 of these cases: 1,331 white males; 825 white females; 2,051 Negro males; and 1,766 Negro females.

The Office of the Coroner in Orleans Parish performs necropsies on cases of homicide, suicide, and accidental or traumatic death occurring in this parish, and upon many patients dying without the attendance of a physician. Particular effort was made in this study to obtain aortas from persons dying suddenly from trauma or poisoning, in which any relationship between the cause of death and the arterial lesions present would be minimized.

Although the aorta was not obtained from every necropsy at either source, there was no conscious bias in selection of material on the basis of the lesions present. In rare instances, the presence of congenital heart disease led to exclusion of the specimen from the series. A few aortas were taken by the surgical staff for aortic homografts during the later years of this study, but there is no reason to believe that these specimens had more or fewer lesions than the others.

The aortas were fixed in 10 per cent formalin, stained for 15 minutes in a 0.5 per cent solution of Sudan IV dissolved in equal volumes of 70 per cent ethanol and acetone, differentiated in 80 per cent ethanol, and rinsed in running tap water for 1 hour. An example of an aorta before (Fig. 1) and after staining with Sudan IV (Fig. 2) is shown for comparison. After blocks were removed for histologic sections, the aortas were sealed in transparent plastic bags.[2] The extent of the gross lesions was estimated by two individuals without any knowledge of age, sex, race, or other clinical data. The specimens have been retained and are available for examination by any interested person.

Three types of lesions were considered: fatty streaks, fibrous plaques, and complicated lesions. "Fatty streaks," as defined in this study (Figs. 3 and 4), represented areas in the intima which stained red with Sudan IV. These may or may not have been appreciably raised above the adjacent intimal surface, but usually the elevation was minimal. Fibrous plaques (Figs. 5 and 6) were raised, pearly, glistening, firm plaques, usually containing material rich in lipid in their depths, but not staining with Sudan IV because of the thick layer of nonlipid hyaline fibrous tissue on the surface. Frequently, small lesions were encountered which appeared to be transitions between

fatty streaks and fibrous plaques, in which the lipid was only partially covered by hyaline connective tissue. The term "complicated lesion" was used to refer to any plaque in which there was additional change, such as ulceration, thrombosis, hemorrhage, or calcification.

The extent of the lesions was recorded as the percentage of the total surface area of the intima involved by each of the 3 types of lesions, as estimated by visual observation. Percentages of surface involvement were grouped into 9 different categories ranging from 0 to 100 per cent. Specimens showing degrees of involvement with fatty streaks representative of each positive category are illustrated in Figures 7 to 14. It was felt that these groups corresponded to the degree of accuracy that might be obtained by visual estimation of highly irregular surface areas.

A percentage value for each type of lesion in the entire aorta was assigned. Then, in order to study the topographic relationships, the aorta was divided into 5 different anatomic regions and an estimate of the percentage of surface involved was again made for each type of lesion in each region. The small scar at the point of attachment of the *ligamentum arteriosum* was graded separately as to the presence or absence of various types of lesions.

Hospital charts and necropsy protocols were reviewed for principal causes of death and pertinent clinical data. These data, together with the results of the grading procedure, were coded and transferred to IBM punch cards for analysis.

Results

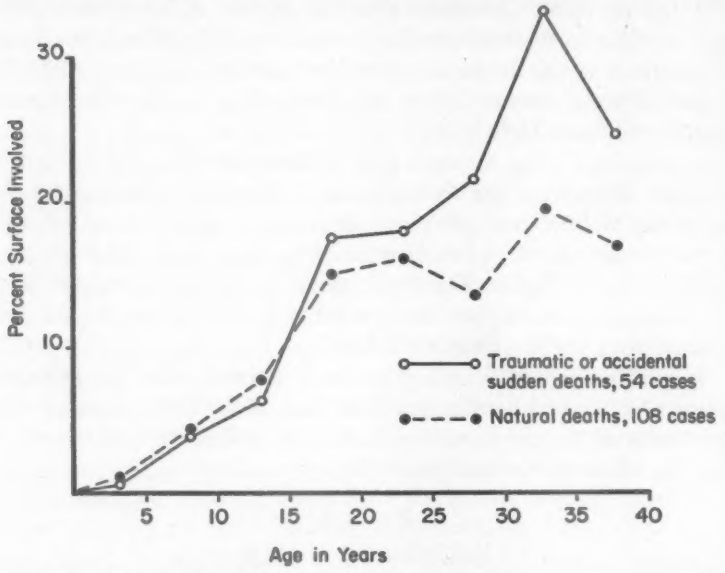
Validation of Methods

Gross Staining With Sudan IV. Gross staining with Sudan IV sharply delineated the intimal fatty streaks, and also made visible those streaks which were not apparent in the unstained specimen. Thus gross staining increased the apparent incidence and extent of fatty streaks in the early age groups. In the present study, sudanophilic material was found in the intima in every case beyond the age of 3 years.

Minimal lesions were found so frequently that some question was raised as to the significance of gross Sudan staining; i.e., whether it actually indicated an early lesion of atherosclerosis. Histologic sections were examined with this particular question in mind, and in every instance there were histologic alterations in the intima corresponding to the macroscopic sudanophilia. These alterations consisted of both intracellular and extracellular globules of lipid (as indicated

by the application of Sudan IV), and a slight increase in interstitial mucinous material. The intima in these early lesions was not always elevated, and there was little fibroblastic reaction.

Estimating Surface Area of Lesions. Visual estimation of the percentage of surface involved by the various types of lesions was tested for reproducibility and reliability by a comparison of values assigned to each of the first 311 specimens by different observers at different times. Over 50 per cent of the aortas were classified in the same

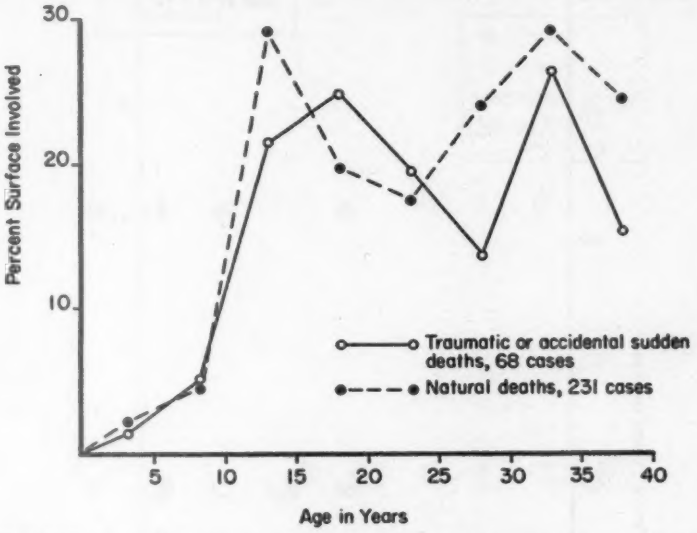


Text-figure 2. Aortic atherosclerosis, New Orleans. Fatty streaks by cause of death in 162 white cases

quantitative category by two different pairs of observers as long as 4 years apart; and only 5 per cent differed by more than 1 category. Agreement was much better between gradings performed by the same individuals at different times. It was considered desirable as a result of the analysis of methods that comparisons should be made on the basis of grading of all the specimens by the same observers. The results reported here are based on data so accumulated.

Sampling of Living Population. A serious question which arose in considering these specimens was how closely the anatomic material obtained post mortem corresponded to the living population. A post-mortem series is a highly selected group, and many factors might introduce bias into the selection. For example, the terminal illness could influence the presence or extent of early lesions. The material

thought to be least subject to any factor which might affect such lesions is that obtained from cases of accidental or traumatic death, and data from such cases were separated and compared with those obtained from the remaining cases. The comparison for fatty streaks is shown for white individuals in Text-figure 2, and for Negroes in Text-figure 3. The apparent differences in white persons after age 25 have been shown not to be statistically significant. Similar comparisons were made with respect to fibrous plaques and to a summation

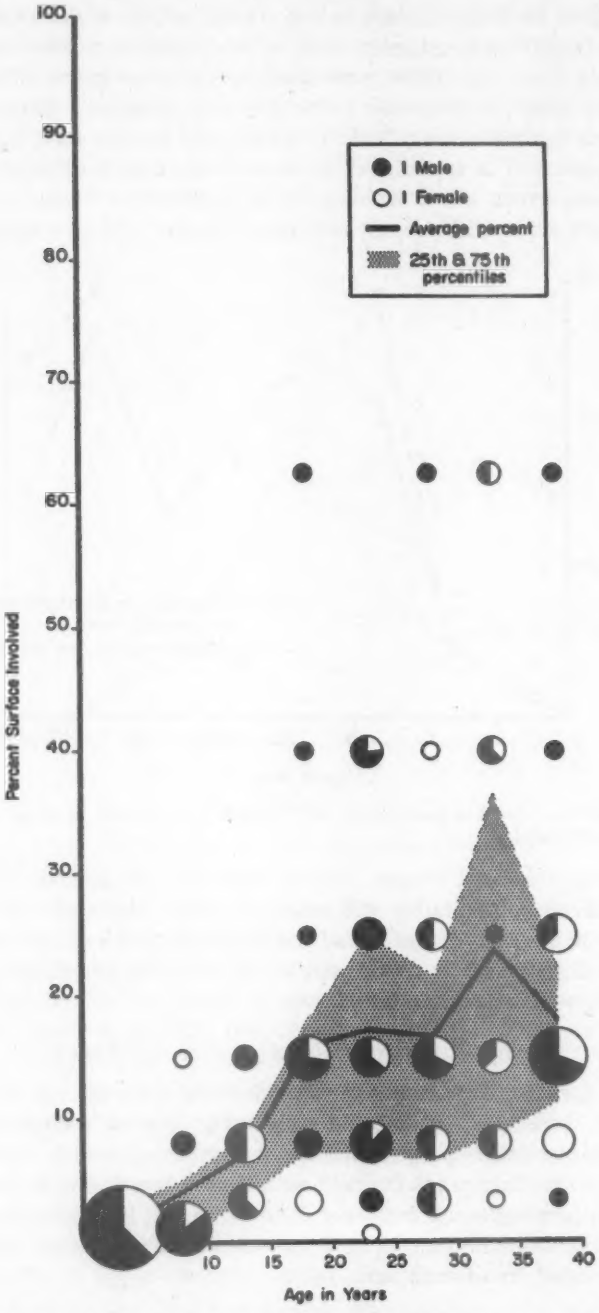


Text-figure 3. Aortic atherosclerosis, New Orleans. Fatty streaks by cause of death in 299 Negro cases.

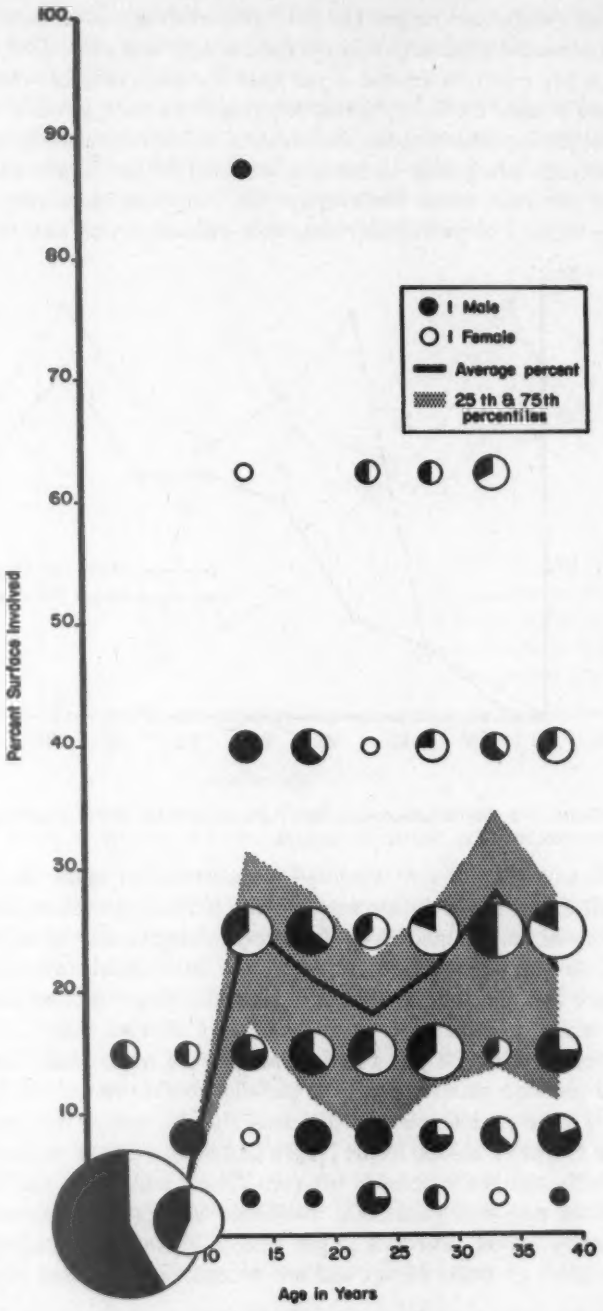
of fatty streaks and fibrous plaques, and the comparisons revealed no significant quantitative difference. It was concluded, therefore, that on the average, the extent of the lesions of aortic atherosclerosis in the entire series was representative of the living population in the community from which it was drawn.

Analysis of Lesions by Age, Sex, and Race

Fatty Streaks. Text-figures 4 and 5 depict graphically the distribution with respect to age, sex, and extent of lesions for each race. The area of each circle is proportional to the number of cases in that category, and the average involvement with lesions together with the 25th and 75th percentiles are indicated. The white and Negro averages by age are compared in Text-figure 6, in which the averages are adjusted to equal numbers of each sex.

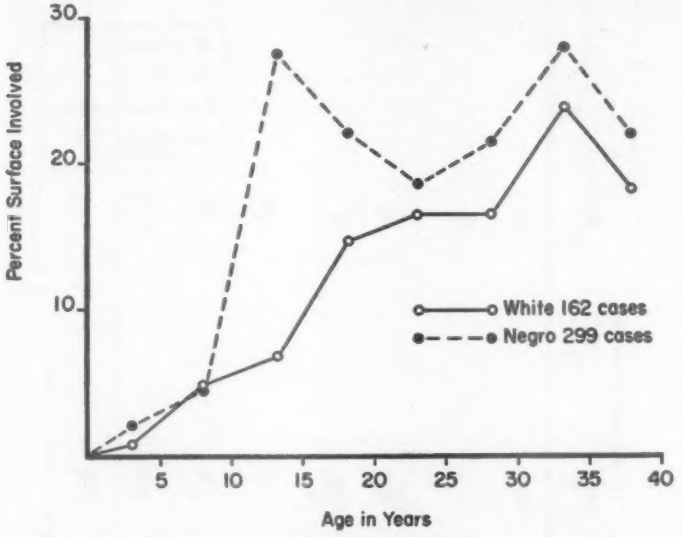


Text-figure 4. Aortic atherosclerosis, New Orleans. Distribution of 162 white cases by age, sex, and per cent of surface covered by fatty streaks.



Text-figure 5. Aortic atherosclerosis, New Orleans. Distribution of 299 Negro cases by age, sex, and per cent of surface covered by fatty streaks.

In both white and Negro, the average percentage of surface involvement proceeded gradually upward from a very low value (0.8 per cent and 2.0 per cent) to around 4 per cent for each race between 6 and 10 years of age. In the statistics covering the next 5 years of age, the most surprising phenomenon encountered in the entire study occurred. The average percentage of surface involved in the Negro cases rose to 28.1 per cent, while the average for the white cases rose only to 7.2 per cent. The probability that this difference was due to chance



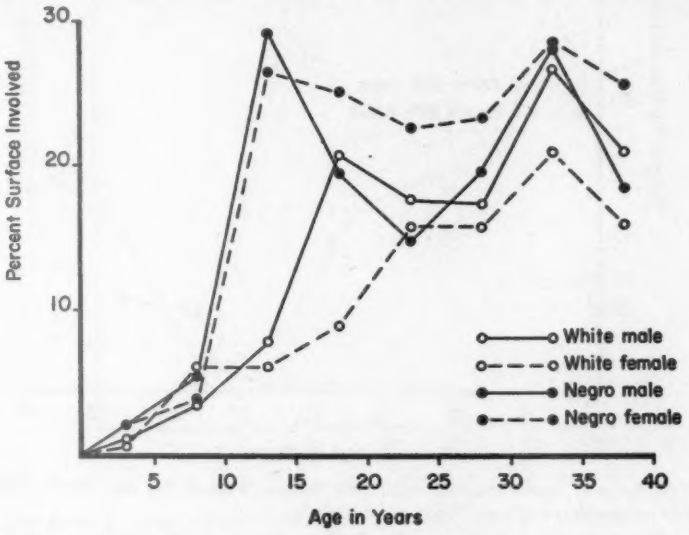
Text-figure 6. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fatty streaks, white vs. Negro, sex adjusted.

alone is less than 0.001. Detailed examination of cases in this age group disclosed no obvious reason for the difference, such as the selection of cases, for the median values corresponded closely to the average values. It was concluded that there is a substantial and significant difference in the extent of lipid streaks in the Negro race as compared to the white race between 11 and 15 years of age.

Between 13 and 18 years of age, among the white cases there was a rapid increase in lesions almost parallel to that noted for the preceding 5-year period among Negroes. By the age of 18 years, the average extent of lesions in the Negro had dropped; and in succeeding years, although the difference between Negro and white was not significant at any one point, the incidence among Negroes remained consistently higher. The fall in per cent of surface involved by fatty streaks after 33 years of age did not necessarily represent regression

of lesions, as will be seen when fibrous plaques are considered, since it may have represented conversion of fatty deposits into fibrous plaques.

When the two sexes were compared without regard to race, no difference in the percentage of surface involved by fatty streaks at any age was seen. That this similarity was artificial, however, was indicated when the sexes were further divided by race as in Text-figure 7. The development of the lesions in the Negro female was much like



Text-figure 7. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fatty streaks by age, race, and sex.

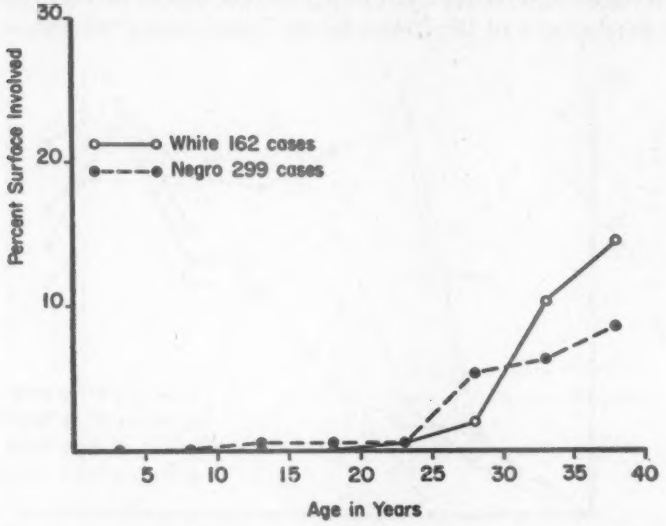
that in the Negro male, except that between the ages of 15 and 30 years in the female there was actually more surface involvement with fatty streaks. On the other hand, the extent of fatty streaks in the white female ranked consistently lower than in all other groups. Thus, the greatest contrast to be seen was that between the white female and the Negro female.

Fibrous Plaques. Fibrous plaques were first observed in cases representing the second decade, and by 20 years of age, 4 Negroes and 1 white in this group had at least minimal fibrous plaques. The frequency with which fibrous plaques were found increased steadily during the third decade of life; and after 30 years of age, 90 per cent of the aortas of each race revealed fibrous plaques to some degree.

The average percentages of aortic surface covered by fibrous plaques are charted for the two races in Text-figure 8. After age 30,

the white was consistently more severely affected, and in the last age group (35 to 40 years), the extent of these lesions in the white was almost twice that in the Negro.

Total Surface Area Affected. The average total percentage of surface covered by both types of lesions was computed by adding the average values for fibrous plaques to those for fatty streaks in corresponding age and race groups. For the whites, the apparent regression of fatty streaks after 36 years of age appeared to be largely accounted for by conversion to fibrous plaques. Attention may be called to the fact that the second stage of rapid progression of the lesions, produced by the formation of fibrous plaques, began in each race approximately 15 years after the period of rapid rise in fatty streaks.



Text-figure 8. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fibrous plaques, white vs. Negro, sex adjusted.

Text-figure 9 shows the average total surface involvement for whites and Negroes, each plotted on the same graph. It may be noted that the total surface involvement among whites over 35 years of age exceeds that among Negroes.

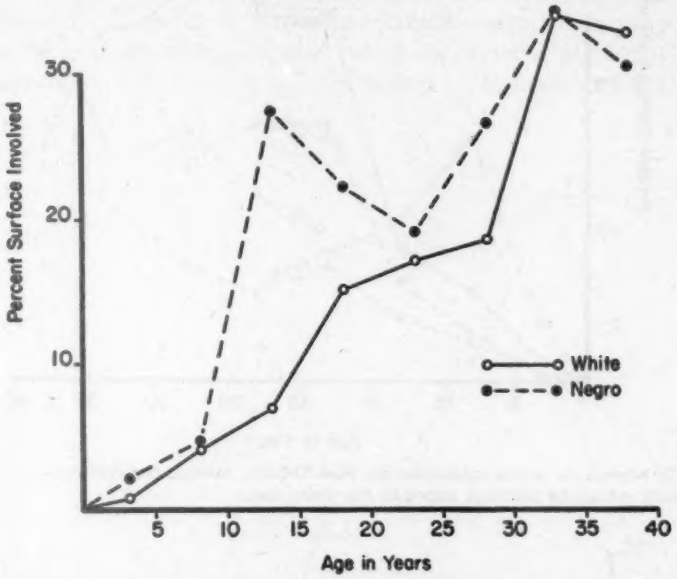
Complicated Lesions. Complicated lesions were quite rare in this group of cases, and quantitatively were insignificant.

Topographical Relationships

The results of the quantitative estimates of the different types of lesions by anatomic region of the aorta are shown for whites in Text-

figures 10 and 11. Similar results were obtained for the Negro race for both fatty streaks and fibrous plaques.

In both races, the aortic ring was the first to be involved by fatty streaks. The sudanophilic deposits encountered in this area were scattered along a line at the upper edge of the valvular commissures.



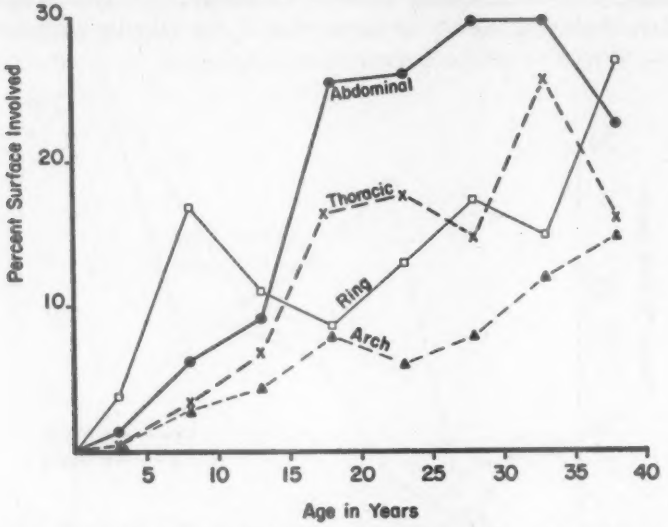
Text-figure 9. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fatty streaks plus fibrous plaques, white vs. Negro, sex adjusted. White, 162 cases; Negro, 299 cases.

The aortic valve leaflets themselves were not affected, and fatty streaks deep within the sinuses of Valsalva were rarely found. Although not sought systematically in this study, fatty deposits were frequently noted on the ventricular surface of the anterior leaflet of the mitral valve.

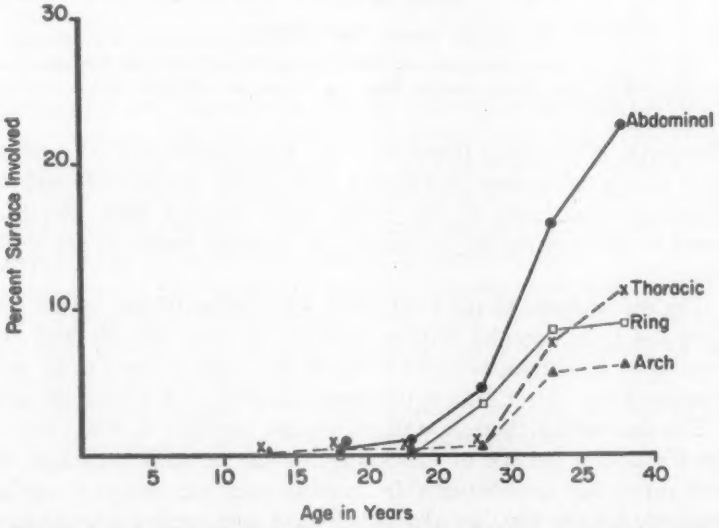
The involvement of the aortic arch was similar to that of the ring. Here the fatty deposits usually occurred in small discrete foci conforming to no particular pattern, except that they seemed to be more frequent about the orifices of the large branches arising from the arch.

The descending thoracic and abdominal portions of the aorta set the distinctive pattern of increasing lesions for each race that has been previously demonstrated in terms of total percentage of surface involved. Of the two, the abdominal aorta was consistently the more severely affected. The same relationship of the various segments of

aorta to one another was also present with respect to fibrous plaques. The abdominal segment was the most severely involved, the descending thoracic was next, and the ring and arch were the least. If the ab-



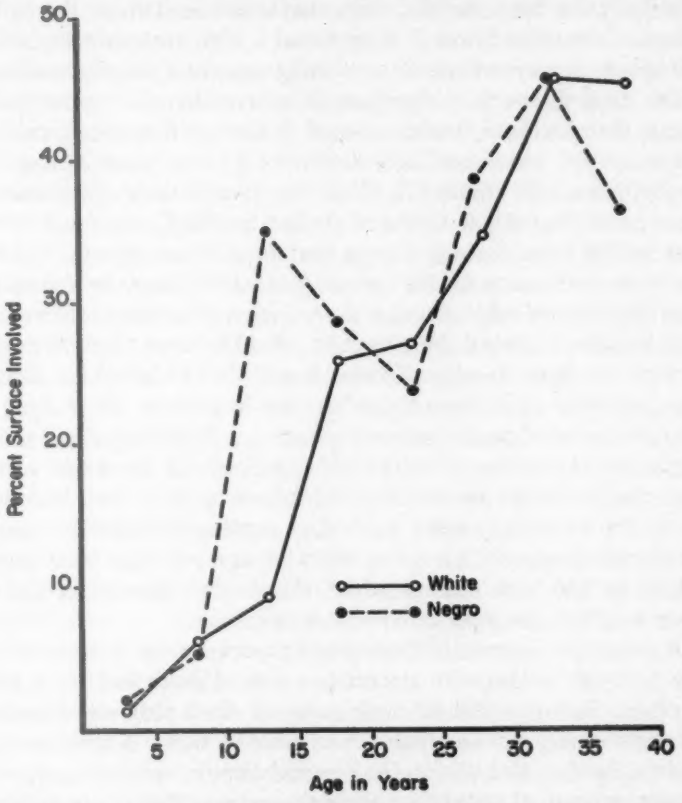
Text-figure 10. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fatty streaks by anatomic region in 184 white cases.



Text-figure 11. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fibrous plaques by anatomic region in 184 white cases.

dominal aorta alone were considered, and if the average percentage of surface covered by fibrous plaques were added to that covered by fatty streaks (Text-figure 12), the same pattern as that shown in Text-figure 6 would be reproduced, with the peaks and the difference between white and Negro at the age of 13 years accentuated.

In the descending thoracic aorta, fatty streaks—especially the extensive ones appearing in the second decade—occurred as parallel, linear, longitudinal streaks localized along the posterior portion of the aorta, between and to each side of the orifices of the intercostal vessels.



Text-figure 12. Aortic atherosclerosis, New Orleans. Average per cent surface covered by fatty streaks plus fibrous plaques in abdominal aorta only, white vs. Negro.

About each orifice there was a small area which was characteristically spared. In contrast, in the abdominal portion the fatty streaks occurred as larger irregular areas, often confluent so as to cover fairly uniformly rather extensive portions of the intimal surface.

Discussion

Concern with the very early lesions of aortic atherosclerosis is by no means new. In 1911, Klotz and Manning[3] introduced their study of the aortas from 90 cases between 1 and 73 years of age with this statement: "It is quite useless to argue the questions concerning the development of intimal scleroses if we study and discuss the late stages of the disease alone. . . . If we wish to gain a true insight into the complex question of arterio-sclerosis we must attempt to follow the lesion from its earliest beginning." Examining their specimens principally for fatty streaks, since they considered these the earliest lesions of "arteriosclerosis," they found a high incidence in individuals below 30 years of age without using any gross staining technique.

The most thorough study of aortic atherosclerosis in younger age groups that has been found reported is that of Zinserling[4] in 1925, who examined 320 aortas from children up to 15 years of age after gross staining with Sudan III. Zinserling graded these specimens into 5 groups on the basis of extent of surface involved, very much as was done in the present study except that he did not attempt quite as precise an estimation of this value. Just as we have, he found that gross staining not only enhanced the detection of lesions in the younger ages, but also increased the estimation of the extent of intimal surface involved by fatty streaks. He also found that sudanophilic deposits were present in some cases before the age of 4 years. In plotting the average degree of aortic lesions against age, Zinserling found only a steady rise in severity of lesions with age, and did not detect a more rapid rise in certain age groups. His failure to do so may have been due to the somewhat cruder method of grading and the fact that he considered cases only up to 15 years of age. As has been demonstrated for the New Orleans white, this is the midpoint of the age group in which the most rapid rise occurs.

Of particular interest in Zinserling's report are the analyses of his data on aortic lesions with respect to cause of death and the state of nutrition. He could find no single cause of death that was associated with significantly more extensive or advanced lesions. A number of the children, having died during the postwar famine, were severely emaciated as a result of starvation; these showed no difference in incidence or extent of fatty streaks as compared with the well nourished ones.

In 1938, Albert[5] reported the results of examination of 136 aortas from patients between 4 months and 25 years of age after Sudan III staining. He could detect no influence of terminal illness on aortic fatty deposits, nor could he detect any clear differences with respect

to sex. Probably for the same reasons as Zinserling, he found no rapid rise in any age period.

The remarkable feature about these earlier studies is the close similarity between the results reported from Europe approximately 30 years ago and those encountered in the present study. The additional conclusions at which we have arrived have been made possible by the larger number of cases, access to material from two races, more precise quantitation, and inclusion of cases up to 40 years of age.

Zeek[6] in 1930 made an exhaustive and critical review of the literature pertaining to juvenile atherosclerosis. Some confusion arose in her discussion because of the inclusion of cases of widespread arterial calcification in very young children, a distinctive entity that seems to be not at all related to the problem of atherosclerosis. After considering many fragmentary studies and a number of conflicting opinions on the subject, she concluded with a series of provocative questions. "Is there a real peak in the incidence of arteriosclerosis around the onset of puberty? If so, why?" Zeek asked. We may now answer with assurance that there is such a peak, and in fact that during this period of life fatty streaks advance more rapidly than at any other period under 40 years of age. Answering "Why?" is more difficult, however. The association of this rapid rise with puberty suggests a relationship to the hormonal changes of puberty.

The fact that this rapid rise during puberty occurs approximately 5 years earlier in the Negro than in the white is one of the best documented and most unexpected results of this study. It is generally recognized that at the present time, the economic status of the Negro in the southern United States is on the average inferior to that of the white. Such an environmental difference would be minimized in the selection of most of the cases studied here, since admission to Charity Hospital is largely restricted to the lower income groups of both races. Precise data concerning the diet of the Negro as compared to the white in this community is not available at the present time, but it is our impression that there exists no real difference in diet paralleling the dramatic difference in lesions in a restricted age group.

The observation that lesions are so nearly universal in young children, in which we have confirmed several previous reports in the literature, has led to serious re-evaluation of the concept of a normal aorta. It means that the existence of a "normal" patient in the sense that his aorta is completely free of structural alterations ordinarily thought of as a disease process does not occur after a very early age; consequently, the population cannot be divided into a group that has

atherosclerosis and another group that does not. All individuals have it, but simply differ in degree of involvement. This suggests that we are dealing with a process which is more or less peculiar to human subjects, the pace of which is set principally by the factors which control growth and development. Atherogenesis may be influenced to some degree in its early stages by environmental factors such as diet, but evidence accumulated in this study points to other stimuli as more important.

While we may have to accept the presence of early lesions of atherosclerosis as "normal" for humans in a statistical sense, we can never accept their presence as "normal" in an ideal sense so long as we believe that it sets the stage for crippling or fatal clinical disorders in later years. Although not every fatty streak inevitably goes through the changes eventually resulting in arterial occlusion, nevertheless the fatty streak appears to be an essential primary step in the series of alterations. Therefore, there is good reason to believe that preventing or reversing fatty streaks would avoid the later stages and thereby prevent clinical manifestations. If this perspective is maintained, there will not arise the difficulty which formerly existed when atherosclerosis was considered an inevitable result of senescence.

We believe that the data presented here are not only consistent with but actually offer considerable circumstantial evidence supporting the concept of pathogenesis described in the introduction (Text-figure 1). For example, it has been shown that there exists a base of fatty streaks sufficiently extensive and persistent to serve as a foundation for all of the fibrous plaques and complicating changes in the lesions which develop in subsequent years. It is difficult to conceive of these intracellular and interstitial lipid deposits resulting from the encrustation or imbibition of fibrin onto or into the intima as has been suggested by Duguid.[7] On the other hand, it appears quite reasonable to suspect that localized areas of tissue injury (manifested as fatty change) might be very susceptible to fibrin deposition and eventual replacement by fibrous tissue, resulting after a long period in a fibrous plaque surrounding a central core of sequestered lipid.

Our data indicate that by the age of 40 years about 20 per cent of the surface involved by fatty streaks has been converted into fibrous plaques; that it requires about 15 years for this conversion to take place; and that fibrous plaques appear in the same proportionate degree in different anatomic regions of the aorta as the fatty streaks did in younger age groups. These relationships, if they continue to hold true as the study is extended to larger numbers and other populations, are significant with respect to any future attempts

to prevent disease due to atherosclerosis. They mean that there is a period of 15 years of life in which lesions are present but are of such a nature that they do not readily produce overt manifestations, and that this period lasts until about the age of 30 years. Until this age, the lesions are almost exclusively of a type that would be much more readily reversible than those appearing later, judging from our experience with fatty degenerations and fibrous proliferative and reparative reactions elsewhere in the body.

The stepwise development of atherosclerosis, implied in the concept of pathogenesis, has also been emphasized by the present study. This feature has already been referred to in discussing the relationship of fatty streaks to fibrous plaques. The other complications occurring in atherosclerotic lesions (particularly those which finally produce sudden arterial occlusion, such as hemorrhage and thrombosis) were rarely encountered in specimens from patients up to the age of 40 years, and it appears that the fibrous plaque must undergo an additional series of changes over a period of at least several more years before the final link in the chain between subclinical lesion and clinical phenomena is forged.

Two apparent paradoxes noted in the data presented are worthy of brief comment and of careful scrutiny in the future as more data become available. The first of these has to do with the greater extent of fatty streaks (Text-figs. 5 and 6) leading to fewer fibrous plaques (Text-fig. 8) in the Negro race. There is no known explanation for this discrepancy, but it does emphasize a point of growing significance to us—namely, that factors responsible for succeeding stages of atherosclerosis (fibrous plaques, complicated lesions, and clinically recognizable disease) may be, and probably are, different from those which initiated the first stage (fatty streak). Intelligent therapeutic attempts must take cognizance of these different stages, for that treatment which is effective in one stage may be ineffective or even contra-indicated in another stage.

The second paradox is the apparent decrease in the extent of fatty streaks in the Negro race between the ages of 13 and 23 years (Text-fig. 5). It is tempting to interpret these data as evidence for reversibility of fatty streaks. It is possible, however, that a wave of environmental factors associated with World War II may have affected Negro children (1 to 5 years old) more than white children. Since the decrease in extent of fatty streaks occurred only in Negro males (Text-fig. 7), it is difficult to see how the privations or imbalances of war should have spared the Negro female. Another observation with similar implications of reversibility is demonstrated

in Text-figure 2, in which it can be seen that cases of natural death have fewer (but not statistically significant in the limited material collected thus far) fatty streaks than those dying of trauma. This suggests that the fatty streaks may have wasted along with other tissues during the terminal illness. As much as we would like to interpret these findings as evidence for reversibility of fatty deposits, prudence justifies patience and more data.

Summary

The aortas from 526 necropsied individuals between 1 and 40 years of age were obtained at a large general hospital and a medico-legal laboratory in New Orleans over a 5-year period. These specimens were examined before and after gross staining with Sudan IV, and the extent of fatty streaks, fibrous plaques, and complicated lesions (hemorrhage, ulceration, thrombosis, or calcification) was estimated for each aorta in terms of percentage of intimal surface affected by each type of lesion. Gross Sudan staining increased the ability to detect fatty streaks, and thus increased both their incidence and extent, particularly in the younger age groups.

Fatty streaks are not precipitated by terminal acute illnesses, for comparison of the average extent of lesions in cases dying suddenly as a result of trauma or poisoning with the average of lesions in natural deaths, discloses no significant difference. It is concluded that this group of specimens is as representative of the living population as it is possible to obtain at the present time.

All patients in this series 3 years of age or older had at least minimal sudanophilic intimal deposits. The percentage of surface involved rose slowly until the age of 8 years, at which time the extent of lesions began to rise precipitously in the Negro. Five years later, the extent of fatty streaks began to rise in the white, but did not reach a peak as high as in the Negro. The patterns in the Negro male and female are very much alike, with more severe involvement in females than in males at some ages. White females were consistently the group least affected.

Fibrous plaques began to appear in the second decade, but did not increase appreciably until the fourth decade. They paralleled the development of fatty streaks, but lagged about 15 years, and the relative degree of involvement of white and Negro was reversed as compared to fatty streaks. By 40 years of age, only about 20 per cent of the area covered by fatty streaks had been converted into fibrous plaques. Additional complications in the lesions were rarely seen in this series.

The aortic ring was the first region of the aorta to be the seat of fatty streaks, but it was the descending thoracic and particularly the abdominal portions which gave the distinctive pattern of increasing lesions between 8 and 18 years. Fibrous plaques also developed most extensively in the abdominal portion.

The data, in general, support the concept of stepwise development of atherosclerotic lesions, and suggest that different factors may be responsible for influencing the various steps in the progression of the lesions.

There is sufficient fatty change to serve as a basis for all the fibrous plaques encountered at later ages, and the data indicate that it requires at least 15 years for the conversion to take place. The rate of development of fibrous plaques and fatty streaks is reversed in the white as compared to the Negro, suggesting that whatever initiates the process differs from whatever carries it on to produce clinical manifestations.

These data do not support the concept of diet as the principal factor in atherogenesis. The rapid rise in lesions during the years of puberty suggests a relationship to the changing hormonal activity encountered during this period.

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We should like to acknowledge the assistance of Byron W. Brown, Jr., Ph.D., now in the Department of Biostatistics at the University of Minnesota, in the statistical analysis of the data presented in this paper.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Unstained aorta from 32-year-old Negro female, dead of carcinoma of the cervix with metastases. Faint outlines of fatty streaks can be seen. Specimen 0-57-749. Reduced to 34%.

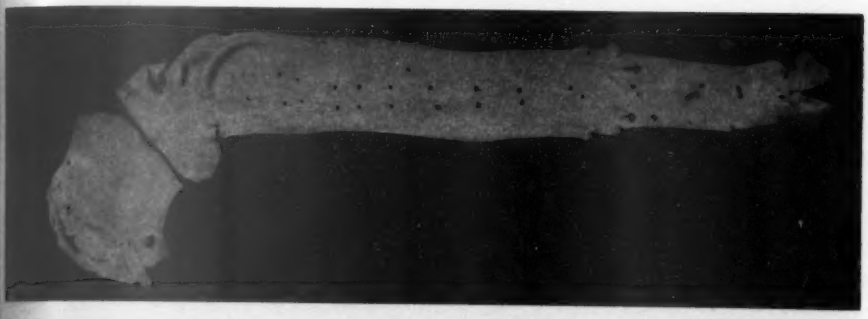
FIG. 2. Same aorta as in Figure 1 after gross staining with Sudan IV.

FIG. 3. Thoracic aorta from a 21-year-old Negro male, dead of acute lymphoblastic leukemia. Stained with Sudan IV, the fatty streaks appear black. Specimen 0-57-724. Reduced to 83%.

FIG. 4. Sudan IV stained frozen section of fatty streak from area indicated in Figure 3. The fat in this photograph appears black and is located both intracellularly and extracellularly. × 400.

FIG. 5. Abdominal aorta of 37-year-old white male, dead of ruptured cerebral aneurysm. The slightly elevated, white fibrous plaques contrast with the fatty streaks which appear black in this Sudan IV preparation. Specimen 1-56-112. Reduced to 83%.

FIG. 6. Sudan IV stained frozen section of fibrous plaque from area indicated in Figure 5. The fat appears black in this photograph. The dense layer of fibrous tissue between the endothelium and the fatty material is responsible for the failure of the lesion to stain grossly with Sudan IV. × 25.



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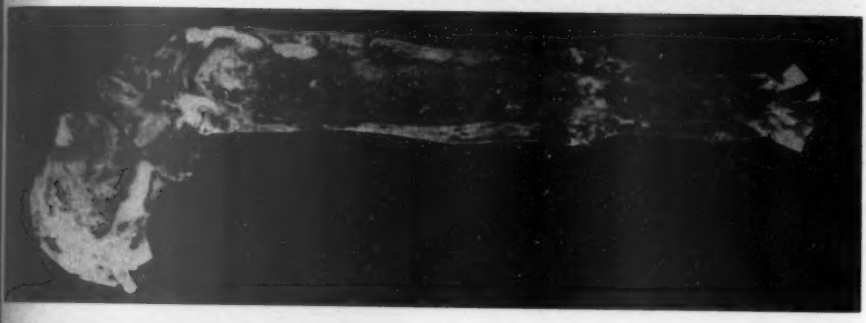

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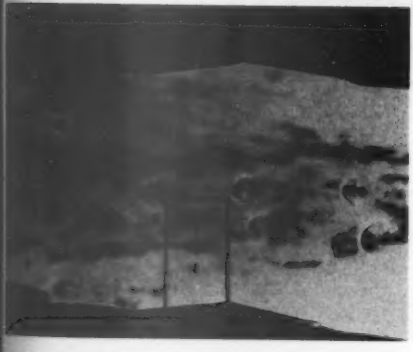

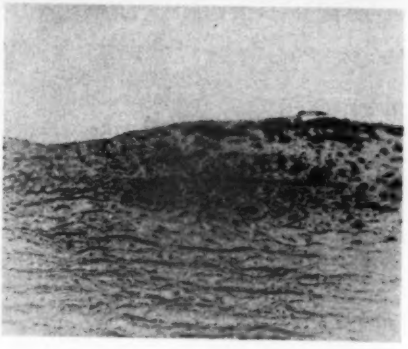

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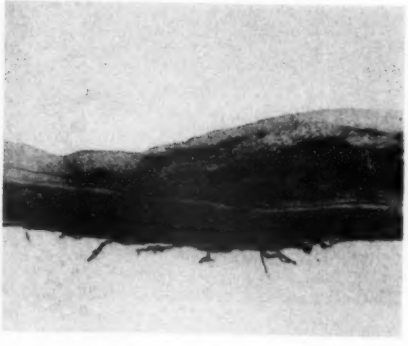

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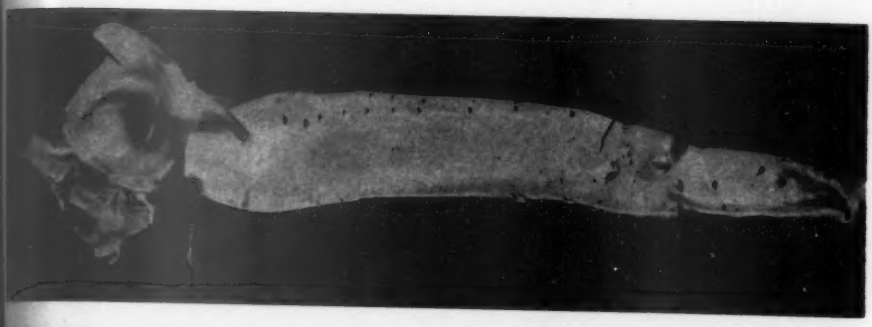
Representative examples of different degrees of involvement of aortic intimal surface by fatty streaks stained with Sudan IV.

FIG. 7. Less than 1 per cent; 4-year-old white male; death due to cardiac arrest during laparotomy for acute appendicitis and peritonitis. Specimen 0-54-1037. Reduced to 58%.

FIG. 8. One per cent to 5 per cent; 8-year-old white male; death due to acute appendicitis and peritonitis. Specimen 1-56-72. Reduced to 50%.

FIG. 9. Six per cent to 10 per cent; 15-year-old white male; death due to shotgun wound. Specimen 1-56-126. Reduced to 38%.

FIG. 10. Eleven per cent to 20 per cent; 17-year-old white female; death due to cobra snake bite. Specimen 1-56-59. Reduced to 45%.



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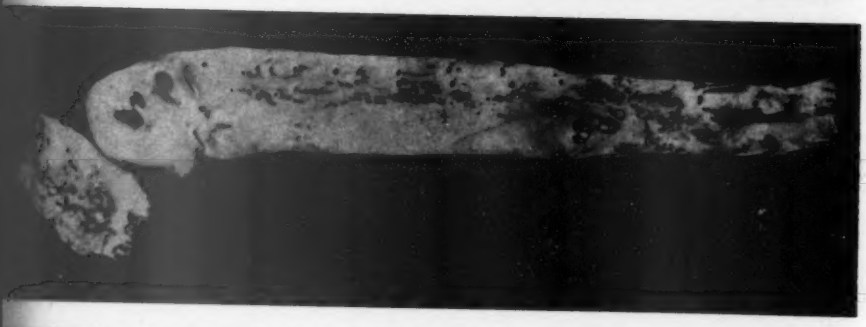

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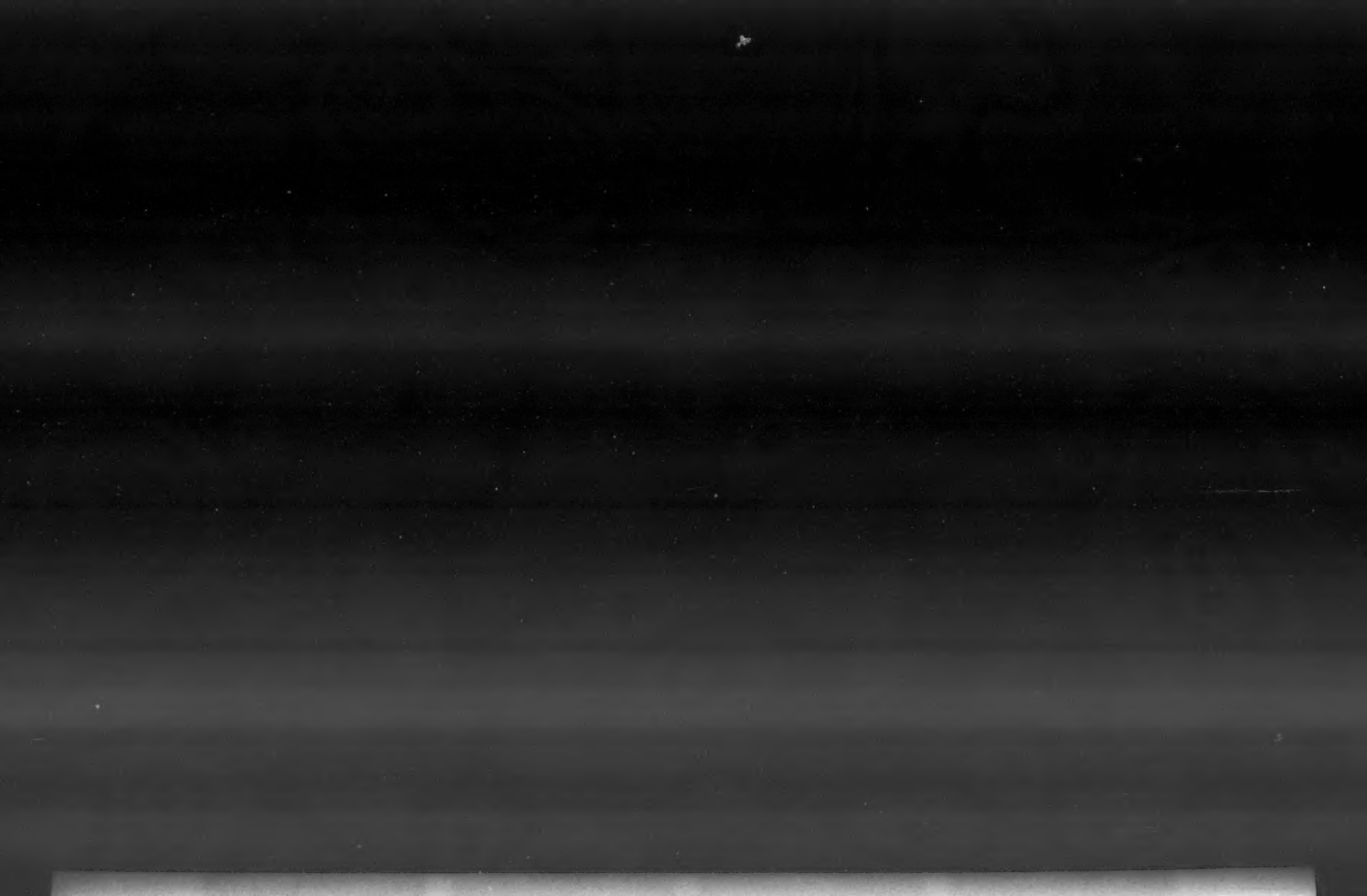
Additional examples of different degrees of involvement of aortic intimal surface by fatty streaks stained with Sudan IV.

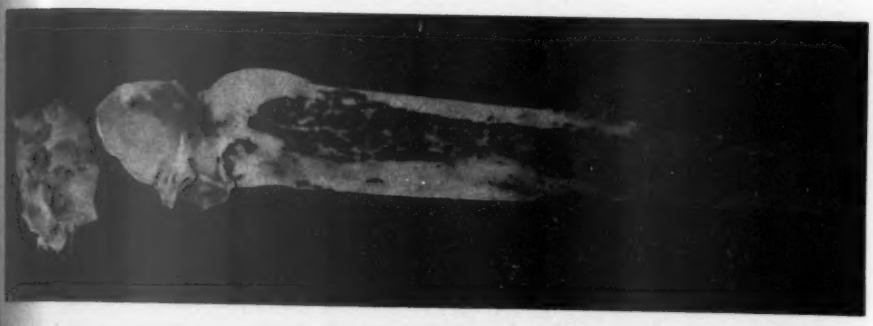
FIG. 11. Twenty-one per cent to 30 per cent; 24-year-old Negro male; death due to suicide by hanging. Specimen 1-56-124. Reduced to 38%.

FIG. 12. Thirty-one per cent to 50 per cent; 25-year-old white male; death due to acute bulbar poliomyelitis. Specimen SBH-56-64. Reduced to 38%.

FIG. 13. Fifty-one per cent to 75 per cent; 38-year-old white female; death due to subacute bacterial endocarditis. Specimen 14.048. Reduced to 38%.

FIG. 14. Seventy-six to 100 per cent; 13-year-old Negro male; death due to tetanus. Specimen 0-55-1664. Reduced to 50%.





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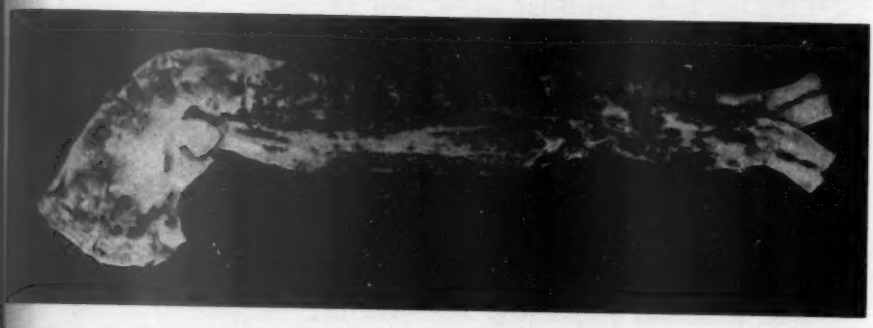

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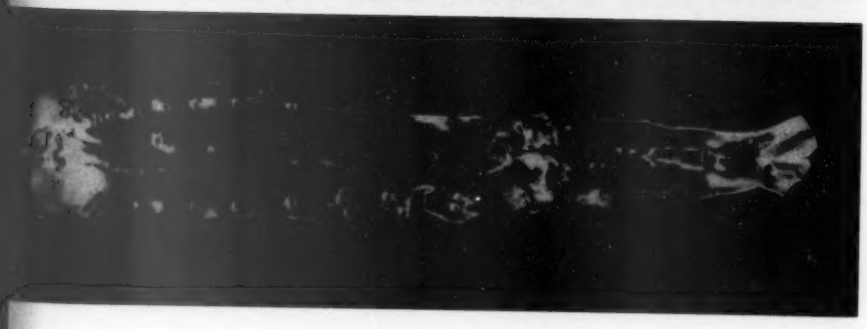

14

THE MORPHOLOGY OF CLUBBING*

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Since antiquity certain changes in the fingers have pointed toward the probable coexistence of serious internal disease. Hippocrates[1] stressed the diagnostic importance of exaggerated curvature of the fingernails in empyema. Aretaeus,[2] in describing the crooked fingernails often found in association with chronic phthisis, attributed the nail changes to a wasting away of all the soft tissues, particularly of the distal nailbed.

According to Ebstein,[3] it was Caelius Aurelianus (*circa* 200 A.D.)[4] and not Hippocrates who first drew attention to the increase in volume of the finger tip as the important feature in clubbing. Except for the brief Hippocratic commentary references to crooked fingernails as a diagnostic sign of empyema and phthisis, there was apparently little interest in these digital changes during the subsequent years until 1832 when Pigeaux[5] published the first definitive work on clubbing.

Bamberger in 1889[6] and 1891[7] and Marie in 1890[8] discovered the presence of bone and joint changes in a few patients with marked clubbing. The latter writer named this "*osteo-arthropathie hypertrophiante pneumique*," and sometimes hypertrophic pulmonary osteoarthropathy is referred to as "Marie's disease." Since these original descriptions of hypertrophic osteoarthropathy, there have appeared several comprehensive reviews on the subject.[3,9-19]

Clubbing and, much more infrequently, the full syndrome of hypertrophic pulmonary osteoarthropathy have been observed not only with certain types of lung disease, but also in association with cardiovascular lesions, extrathoracic abnormalities, and as a primary familial or nonfamilial disease unaccompanied by other disorders.[15] Although classically important in the diagnosis of bacterial diseases of the lungs, the development of surgical techniques for pulmonary resection has provoked more and more interest in the study of clubbing because of the frequent association of clubbing with carcinoma of the lung.

The clinical findings[3,8-19] and roentgenologic picture[13,15,17,20-23] have been thoroughly described in clubbing and hypertrophic osteoarthro-

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pathy. The histologic changes in the skeletal system have been reported in detail by Crump[24] and others.[25-29] There is, however, some lack of clarity as to the structural alterations involved in clubbing. With respect to the pathogenesis of this disease, much remains to be learned, and we are today reminded of Samuel West's comment in 1897: "Clubbing is one of those phenomena with which we are all so familiar that we appear to know more about it than we really do."[30]

Definition and Clinical Description of Clubbing

Clubbing is characterized by a bulbous enlargement of the distal segment of a digit. This digital segment may approach spherical form in marked clubbing. Toes are usually affected as well as fingers, except in cases of unilateral clubbing. But the deviations from normal in toes are more difficult to ascertain because of the effects of shoes and posture.

Lovibond[31] was the first to publicize objective measurement of the "profile sign" as the criterion for clubbing. When a nonclubbed digit is flexed at the distal interphalangeal joint, it is observed that an angle is formed by the following two lines drawn in the midsagittal plane: a line parallel to the nail at the nail base; a line resolving the surface contour of the soft tissue proximal to the nail on the dorsum of the distal phalanx. The apex of this angle normally points toward the palmar side of the digit, and the angle is equal to approximately 160°. The essential change in clubbing is an increase of this angle, approaching a straight line. In exaggerated clubbing, this angle may be greater than 180° and the point of union of nail and soft tissue at the nail base projects dorsally.

The beginning stages of clubbing can be detected by palpation at the base of the nail. In early clubbing the nail becomes more freely movable. The examiner notes a spongy sensation as though the nail is being ballotted on an edematous pad. The skin over the nail base is smooth and shining; the minute skin creases disappear. Color changes are not constant, but cyanosis of the nailbed and increased pink hue of the skin at the nail base are often observed. The process of clubbing is usually painless and so gradual as to proceed unnoticed by the patient. An exception to this is sometimes the clubbing occurring with lung carcinoma, which may be abrupt in onset and associated with pain and difficulty in moving the fingers.

Summary of Previous Descriptions of Clubbing

Pigeaux in 1832[5] wrote that blood-tinged fluid infiltrated the soft tissues under the nail and thus elevated the nail. However, he could see no changes microscopically. Several reports in the next 80

years[25,26,32-36] were remarkable chiefly in their variance with one another over the histologic description of the clubbed digit.

Grafe and Schneider (1913)[37] concluded that the chief reason for abnormal curvature of the nail was increased thickness of the nailbed. Locke reported in 1915[13] that there was some edema of the cutis and thickening of arterial walls. In 1923 Schirmer[38] reported the unique finding, in the subcutaneous tissue, of a net of fine connective tissue fibrils with a homogeneous basophilic ground substance in which many capillaries and a few infiltrations of round cells were seen. The homogeneous ground substance gave a reaction with thionine and mucicarmine in a manner consistent with mucin. Schirmer postulated that clubbing might be due to an embryonal mucinous substance replacing the adult type of subcutaneous connective tissue.

Campbell in 1924[39] published descriptions and photomicrographs of one clubbed and one normal finger. He pointed out that the essential difference between the two was edema of the tissues of the finger tip, especially in the nailbed. In 1950 Bariety and Coury[17] noted capillary dilatation and edema as the sole pathologic changes.

Lovell (1950)[40] examined 5 clubbed and 2 normal fingers fixed and stained by an intra-arterial injection technique. In the clubbed fingers the nailbed was thicker than in the normal state; this was due to an increase in connective tissue. Less obvious was the increased thickness of the fibrous capsule enclosing the pulp. In a clubbed specimen from a patient with a congenital heart disease the superficial venous plexuses were conspicuously dilated, especially in the nailbed. The injection of neoprene into the blood vessels and clearing by the Spalteholz technique showed no difference in vascular filling among 4 normal and 3 clubbed specimens.

Gall, Bennett and Bauer (1951)[29] described the pathologic features of clubbing in connection with a study of the bone and joint changes in generalized hypertrophic osteoarthropathy. It was reported that the collagen bundles in the digits were swollen. Soft tissues were infiltrated by lymphocytes and plasma cells. The periosteum showed only minimal change—a division into outer fibrous and inner cambium layers.

Schoenmackers in 1956[41] studied clubbed toes from 26 cases of cyanotic heart disease, 5 cases of mitral stenosis, and 22 controls. He confirmed the increased thickness of the nailbed. In the nailbed sections were foci of increased translucency where elastic and collagen fibers were not discernible. In some cases there were markedly congested venous sinuses. In a few specimens the glomuses showed definite enlargement.

The published reports on the structural alterations of clubbing agree in there being no change or only minimal modifications in the bony structure of the terminal phalanx. Soft tissue changes have been held responsible for the digital enlargement. Edema, increase in connective tissue, arterial hypertrophy, capillary dilatation, and mild chronic inflammation have been noted in most of the more recent publications. The need for a comprehensive study of clubbing is made apparent, however, by the contradictions in the previous descriptions and the limited number of reports. In fact, a single specimen was examined in most of the studies and (except in 3 instances) explicit reference to control material was not made.

Methods

The hands and feet of all 350 patients examined at necropsy at the Institute of Pathology of the University of Munich during a 4-month period were investigated for the presence of clubbing. The degree of clubbing, when present, was estimated in arbitrary grades I to V. Grade I denoted increase of the "profile sign" angle to between 160° and 180°. Clubbing of grade II severity existed when the nail and proximal soft tissue surface were in a straight line. In grade III the dorsal angle at the junction of the nail and soft tissue was slightly greater than 180°, but the circumference of the distal phalangeal segment was approximately equal to that of the next proximal segment. Grade IV denoted an angle greater than 180° with the ratio of the diameter of the terminal phalanx to the diameter of the proximally adjacent phalanx slightly greater than unity. In grade V there was marked protrusion of the angle formed by the nail and the soft tissue at the nail base. There was also bulbous enlargement of the finger tip so that the diameter of the terminal segment was markedly greater than that of the proximally adjacent segment.

Ten instances of clubbing were found, and 29 cases without clubbing were selected as controls. With the exception of a 7-month-old child, the controls ranged in age from 21 to 74 years (6 cases being in the third decade, 5 in the fourth, 3 in the fifth, 6 in the sixth, 7 in the seventh, and 1 in the eighth). Fourteen patients were male and 15 female. Table I lists the cases with clubbing.

The specimen obtained in each case consisted of the entire terminal segment of the left thumb, devoid only of the nail and the skin except for a longitudinal strip 0.5 cm. wide on the palmar aspect where the epidermis lay *in situ* over the entire length of the specimen. All specimens were subjected to the same treatment: fixation for 2 weeks in Müller's formalin (10 cc. of 40 per cent formalin mixed with a solution

of 2.5 gm. $K_2Cr_2O_7$ and 1 gm. Na_2SO_4 in 100 cc. of H_2O); washing; demineralization in 5 per cent HNO_3; cutting of a thin midsagittal block; neutralization in 5 per cent Na_2SO_4 solution; washing; embedding in celloidin. Midsagittal sections were cut at 15 to 20 μ. Sections from all 39 specimens were stained with hematoxylin and

TABLE I
Cases With Clubbing

Case	Age	Sex	Diagnosis	Grade of clubbing	Thickness of nailbed
1	36	F	Chronic empyema Tuberculosis Tertiary syphilis	I	2.0 mm.
2	52	M	Rheumatic heart disease (marked aortic stenosis)	I	
3	9 mo.	M	Congenital heart disease (cyanotic type)	II	1.2 mm.
4	59	M	—	II	2.5 mm.
5	33	M	Bronchiectasis Chronic pleuritis	III	2.5 mm.
6	35	M	Hodgkin's disease (pulmonary involvement)	III	3.0 mm.
7	35	M	Chronic empyema	IV	4.0 mm.
8	51	M	Marked emphysema and bronchitis (asthma) Cor pulmonale	IV	2.5 mm.
9	27	M	Bronchiectasis Brain abscess Purulent meningitis	V*	
10	38	M	Chronic lung abscess Bronchiectasis Cor pulmonale Syphilitic meningitis	V	

* With hypertrophic periostitis of tibia, fibula, radius and ulna.

eosin. In addition, microsections of the clubbed specimens and 5 controls were stained by Verhoeff's method for elastic fibers[42] and with Alcian blue for the demonstration of mucin.[43] The last named staining technique is not specific for mucin since chondroitin sulfuric acid complexes as well as mucoitin-sulfuric acid complexes combine with the dye.

RESULTS

The thickness of the nailbed varied from 1.0 to 1.7 mm. in the control specimens except for 3 cases where the thickness was 2.0 mm. from the nail to the bone. Figures 4 and 6 demonstrate another ob-

servation when the sections were viewed without the aid of a microscope. There was increased translucency in the proximal half of the nailbed with the small blood vessels standing out in bold relief. The periosteum of the nailbed was also thicker in the clubbed specimens than in the controls. However, there was no gross change in the structure of the bone and soft tissues on the palmar side of the phalanx.

Microscopically all the clubbed specimens but one showed a less dense organization of connective tissue in the proximal nailbed (Fig. 8). The collagen fibrils and cells were separated by a greater distance. The fibroblasts (Fig. 10) appeared more primitive, exhibiting large nuclei, abundant faintly basophilic cytoplasm, and long reticular processes. The overall scarcity of formed elements with large fibroblasts set in a reticular network was most characteristic of the regions of translucency noted on gross examination, but this appearance extended to the distal portion of the nailbed. Here there was increased widening of the interfascicular spaces even in places where there were thick, dense collagen fibers.

In most of the clubbed specimens there were more lymphocytes scattered extravascularly through the nailbed than in the controls. In clubbed specimens from cases 7 (Fig. 11) and 9 there were focal perivascular accumulations of lymphocytes. In half the cases there was a moderate increase in the number of tissue eosinophils. It is noteworthy that the increased numbers of lymphocytes and eosinophils occurred only in the nailbed and not in the soft tissues on the palmar side of the phalanx.

The periosteum of the nailbed was thickened in the more severely clubbed specimens. Penetrating deep into the periosteum were small blood vessels immediately encompassed by loose reticular tissue. Extravascular lymphocytes were observed in these projections. Four of the cases with clubbing demonstrated an abnormality in the periosteum on the palmar side of the bone. One or two small collections of loosely textured connective tissue similar to that of the nailbed were found between the cambium and fibrous layers of the palmar periosteum (Fig. 12).

The arteries in the nailbed were of larger size than those in the controls, but the increase in size appeared only commensurate with the increase in soft tissue of the nailbed. In sections from clubbed cases 4 and 7 there was glomus formation throughout the length of the nailbed. The vessels, appearing to be arteriovenous anastomotic units,[44,45] were of increased caliber and number (Fig. 14). The vascular elements within a section of a glomus were also more widely separated because of the widening of the interstitial spaces.

The specimen from clubbed case 8 (Fig. 5) was an exception to

the pattern evident in the other specimens. The corium of the palmar aspect was increased approximately 0.5 mm. in thickness when compared to the other clubbed specimens and controls. This increase in thickness was caused by the presence of an abnormally thick, dense mat of collagen fibers. Other abnormalities were restricted to the nailbed. In this region there was no widening of the interfascicular spaces. The connective tissue was very compactly organized. The entire nailbed had increased collagen deposition. The vascular components of the glomuses were thick-walled and encased in a thickened fibrous sheet. There was no increase in the number of extravascular lymphocytes or eosinophils.

Table II summarizes the individual observations and shows the rather good correlation among increased thickness of the nailbed, extent of interstitial edema of the nailbed, and the clinical grade of clubbing.

TABLE II

The Anatomic Changes in Clubbing

		Nailbed					Periosteum
Case	Grade of clubbing	Increased thickness	Edema	Increased no. of lymphocytes	Increased no. of eosinophils	Increased no. coils A-V anastomoses	Nailbed-type tissue on palmar side
1*	I	+	+	+	+	o	o
2	I	+	+	o	o	o	o
3	II	+	+	o	o	o	o
4	II	+	+	†	o	+	o
5	III	+	++	+	+	o	+
6	III	++	++	+	++	o	+
7	IV	+++	+++	+++	o	++	o
8‡	IV	+	o	o	o	o	o
9	V	+++	+++	+++	+	o	+
10	V	§	+++	+	+	o	+

* Endostosis.

† Accumulations of lymphoblasts.

‡ Dense collagen in nailbed, palmar corium, fatty tissue septa.

§ Part of nailbed was inadvertently removed.

Few controls showed the abnormalities described in the clubbed specimens. Increased thickness (to 2.0 mm.) and looseness of texture of the nailbed with widened interfascicular spaces and large fibroblasts in a fibrillar network were present in the case of a 29-year-old female with sarcoma in the mediastinum and bilateral pulmonary infiltrations. The microscopic structure of the nailbed was similar to that in clubbing of grade I severity. No control possessed the loosely textured tissue on the palmar side of the phalanx described in clubbed cases 5, 6, 9 and 10, or any increase in number and size of arterio-

venous connections in the plane of the section as in clubbed cases 4 and 7. A moderate increase in the number of diffusely distributed extravascular lymphocytes in the nailbed was observed in a 40-year-old male with carcinoma of the stomach. There was also a slight increase in the number of tissue eosinophils scattered through the proximal half of the nailbed in the specimens from the following controls: a 27-year-old male with chronic caseous pulmonary tuberculosis and chronic empyema; a 30-year-old female with thrombocytopenic purpura secondary to aminopyrine sensitivity; and a 51-year-old male with complications of arteriosclerotic coronary artery disease.

Since there have been several reports attributing the changes in clubbing to hypertrophy or edema of all the soft tissues of the digit, 2 cases were studied in which each of these changes was observed upon gross examination. Specimens from two manual laborers with digital hypertrophy showed neither increased thickness nor microscopic abnormality of the nailbed. In one of these (Fig. 2) there was increased thickness of collagen in the palmar corium and some displacement of the fatty tissue by dense connective tissue on the palmar side of the distal phalanx; in the other, the hypertrophy was due to increase of all tissue components without distortion in the pattern of arrangement except for hyperkeratosis.

Of 2 cases with generalized anasarca including edema of the digits, one had nailbed thickness of 2.0 mm. with the only microscopic abnormality being swelling of the collagen bundles in the septa of the fatty tissue layer on the palmar side of the phalanx. In the other (Fig. 3) the nailbed was 1.7 mm. thick. There was a slight increase in the width of the interfascicular spaces of the nailbed, but the large fibroblasts of the clubbed specimens were lacking.

The stain for elastic tissue confirmed the findings made with hematoxylin and eosin: there was no change in the distribution of elastic fibers. The clubbed specimens and all 5 of the controls tested gave the same reaction when subjected to the Alcian blue staining technique for the demonstration of mucin and chondroitin. The articular cartilage at the interphalangeal joint was stained, the intensity varying inversely with the distance from the joint cavity. Bone showed no affinity for the dye. The soft tissues combined minimally and uniformly with the stain.

Discussion

Changes in the nailbed were responsible for the alteration in size and configuration of the clubbed digit in this study. Increased thickness of the nailbed was noted in every case. Clubbing was present

when the thickness of the nailbed exceeded 2.0 mm. This thickness was due largely to interstitial edema in all but one case. Increase in extravascular lymphocytes was observed in the majority of cases; in 2 cases there were several dense aggregations of these cells. Increase in the number of tissue eosinophils was more inconstant and less striking. The periosteum on the palmar side of several clubbed specimens exhibited a prevalence of the loose-textured connective tissue characteristic of the nailbed.

The alteration in the glomeral pattern of the nailbed in cases 4 and 7 was of particular interest. In the plane of the section there were more arteriovenous anastomotic units transected than in the control specimens. Comparison of Figures 13 and 14 illustrates this abnormality. Unfortunately, it was impossible from the material at hand to quantitate this observation adequately. It was quite evident on study of the sections that, as Popoff[44] has emphasized, the glomuses are most numerous in a plane parallel to the skin or nail surface. In a random section perpendicular to this plane, however, the sampling error does not permit valid conclusions from the present study other than to note that glomeral patterns in 2 cases of clubbing suggested increased number and size of the arteriovenous connecting vessels. The glomeral structure warrants further study, utilizing sections made tangential to the nail and skin surfaces.

Case 8 showed a more chronic form of clubbing. In this stage, edema did not appear to be part of the lesion. Cellular infiltrations were also absent. Increase in volume was due to increased deposition of dense collagen in the nailbed, palmar corium, and fatty tissue septa.

That the digital changes in clubbing are different from those seen with generalized edema of the hands or hypertrophy associated with hard manual labor is evident. The localization of the interstitial edema to the nailbed, and the other changes noted are unique to the disorder of acute clubbing. The questions arise: Why are the changes found in this site? What is the factor or combination of factors causing the predilective involvement of the nailbed in clubbing?

The proximal portion of the nailbed is metabolically active in the elaboration of the fingernail. Another noteworthy characteristic of the nailbed is the abundance of glomus formation in this tissue.[44,45] It has been reported that the number of anastomoses per square centimeter of surface area in one digit studied was 510 in the nailbed, 236 at the finger tip, and 150 in the palmar tissue of the distal phalanx.[45]

It was particularly interesting to note in the thickened periosteum of the phalanx, interfascicular edema and small collections of the same type of loosely arranged connective tissue encountered in the nailbed.

Crump[24] described regions containing sparse cells and fibrils in the periosteum of bones showing hypertrophic periostitis. An increase in cells with oxyphilic granules was noted in some of these foci. Gall and co-workers[29] described edema as a prominent feature of the early periosteal change associated with subperiosteal new bone formation. Of importance is this morphologic similarity of the periosteum in the process of clubbing and in hypertrophic periostitis.

Theories of Pathogenesis

There is an abundance of speculation concerning mechanisms of clubbing in previous publications. Many reviews provide lengthy theoretical discussions which do not require repetition. Certain recent developments, however, merit attention.

One of the handicaps in the study of the pathogenesis of clubbing and hypertrophic periostitis, despite numerous attempts to produce this pathologic state experimentally, is the dearth of positive results.[7,46-51] Mendlowitz and Leslie[48] anastomosed the left pulmonary artery and left atrium in the dog, producing a right-to-left shunt with corresponding desaturation of systemic arterial blood. It was shown that in addition to cyanosis these dogs had an increase of cardiac output after the shunting procedure. One dog so treated developed minimal but demonstrable changes of hypertrophic periostitis on certain long bones.

The influence of certain surgical procedures on soft tissue, bone and joint changes in hypertrophic osteoarthropathy associated with carcinoma of the lung has been reported recently. Excision of the affected pulmonary tissue,[19] ligation of the pulmonary artery,[52] section of the nerve branches at the hilus,[53,54] and severing of the vagus nerve just below the recurrent laryngeal nerve[55] on the affected side, have all been reported as bringing about prompt and dramatic remission of the peripheral signs and symptoms.

Mendlowitz[18] has recently summarized his studies on the abnormal physiology of the digital circulation in clubbing. He has found by calorimetric measurement that acquired symmetrical clubbing is usually associated with increased blood flow in the finger tips, this increase in blood flow being out of proportion to the increase in soft tissue mass. Mendlowitz[18,56] has propounded the theory that in clubbing there is an increase in pulmonary circulation without a corresponding increase in the demand for blood in the systemic circulation. This produces a local oversupply of blood to the fingers and toes, and—in cases of osseous involvement—to the affected bones. Questions unanswered by this theory are: (1) What causes this increase in pul-

monary circulation, if it does exist? (2) What is the mechanism whereby digital blood flow is increased? The function of the arteriovenous anastomoses in the glomuses is presumably involved, but how is this effected?

Summary and Conclusions

Midsagittal sections of the thumb from 10 cases of clubbing and 29 nonclubbed control specimens were examined. The alteration in shape of the digit in clubbing resulted from an increase in thickness of the nailbed. In the series of cases studied, a thickness of the nailbed of the thumb greater than 2.0 mm. was found only in clubbed digits. The nailbed was loosely textured with large primitive-appearing fibroblasts in a wide-meshed reticular network. There was often an increased number of extravascular lymphocytes and eosinophils. In 2 cases the glomeral structure in the nailbed was altered, with more coils of arteriovenous anastomoses than usual. Severely clubbed specimens commonly showed foci of tissue in the periosteum on the palmar side which resembled the tissue in the nailbed. In addition there were edema and thickening of the periosteum. Review of the histologic observations of hypertrophic periostitis in previous writings revealed that in the periosteum overlying altered bone there had been reported focal edema and loose-textured connective tissue similar to that seen in the nailbed in this study.

In one case of chronic clubbing no evidence of edema was seen. The increased thickness was due to increased collagen deposition in the nailbed and corium of the palm.

Further study of the subungual glomus in clubbing is indicated. The similarity of the periosteal lesions in clubbing and hypertrophic osteoarthropathy points toward a similarity in pathogenesis. The new formation of bone observed in the long bones in the latter condition, however, has not been observed in the nailbed in simple clubbing.

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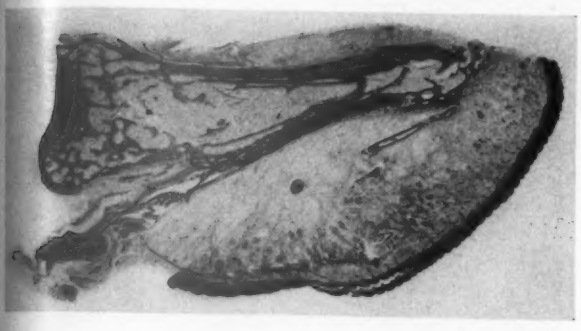
LEGENDS FOR FIGURES

All photographs are of sections stained with hematoxylin and eosin.

FIG. 1. Midsagittal section through the terminal phalanx of the left thumb from a nonclubbed control. Forty-year-old male with carcinoma of the stomach, and metastasis. × 3.

FIG. 2. Nonclubbed thumb from a 43-year-old male manual laborer who died with periportal fibrosis of the liver and ruptured esophageal varices. Thumb shows hypertrophy of all tissue components. × 3.

FIG. 3. Grossly edematous thumb from a 61-year-old female with edema of all extremities secondary to starvation hypoproteinemia. Except for osteoporosis, there is essentially no variation in the section from the other control cases. × 3.



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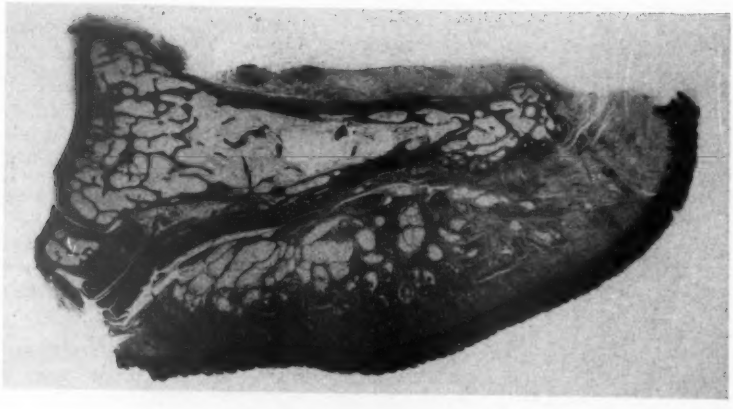

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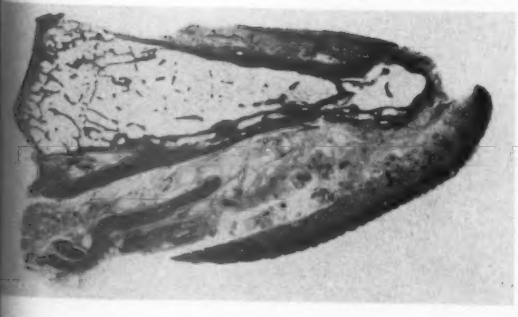

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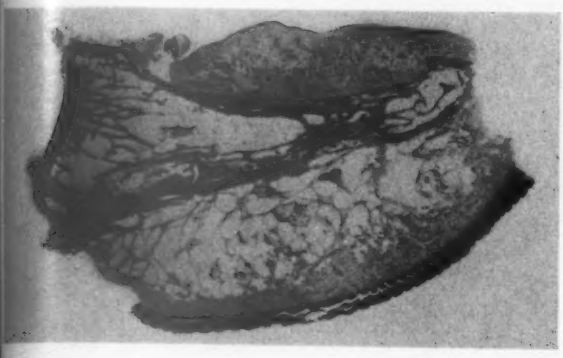
FIG. 4. Thumb from case 7, grade IV severity of clubbing. Thirty-five-year-old male with chronic empyema. Note marked increase in thickness of nailbed, increased vascularity and increased translucency of proximal nailbed. × 3.

FIG. 5. Case 8, grade IV clubbing. Fifty-one-year-old male with long history of asthma. Necropsy examination showed marked emphysema and bronchitis, chronic cor pulmonale. Midsagittal section of thumb reveals increased thickness and density of nailbed. There is increased thickness of dense connective tissue subcutaneously and of fibrous septa in fatty tissue layer on palmar side of the bone. × 3.

FIG. 6. Case 9, grade V clubbing. Twenty-seven-year-old male with bronchiectasis. Changes present are increased thickness of nailbed, decreased density and increased vascularity of proximal and middle portions of nailbed. Small region of tissue similar to that of the nailbed is seen in palmar periosteum at junction of middle with distal third of phalanx. × 3.



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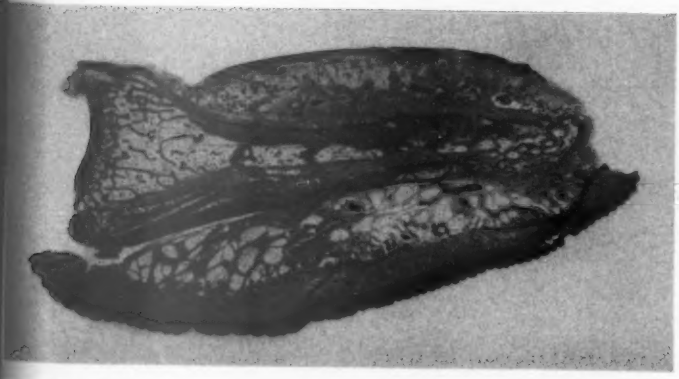

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FIG. 7. Midsagittal section of thumb (control specimen) from a 32-year-old female who died with chronic glomerulonephritis. Middle portion of nailbed with bone on the left. × 72.

FIG. 8. Clubbed case 7. Middle portion of nailbed with periosteum out of view to left. Apparent are the increased thickness of the nailbed, relative hypocellularity, widening of the interstitial spaces, and—in the center of the picture—the large fibroblasts. × 72.

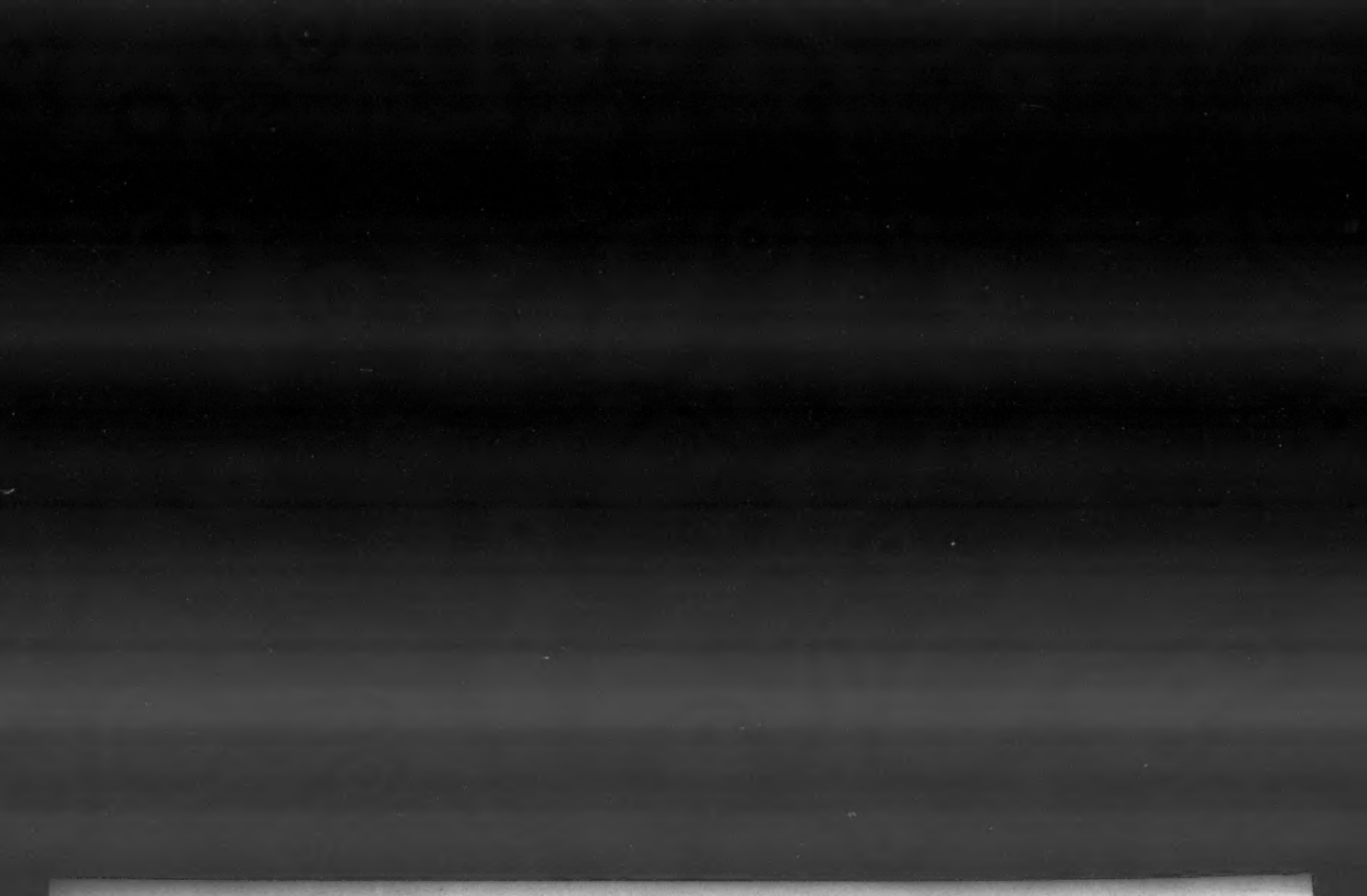

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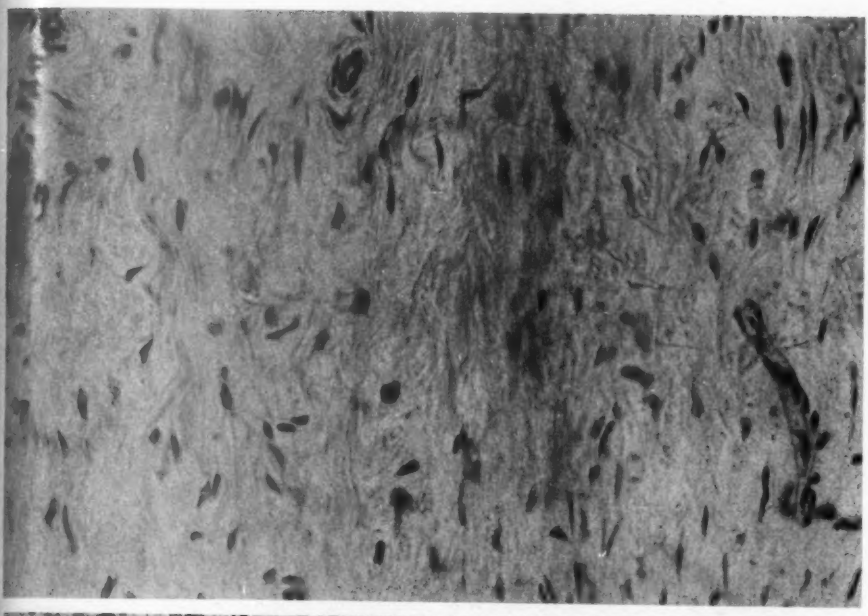
FIG. 9. Higher magnification of Figure 7. Nonclubbed control. × 300.

FIG. 10. Higher magnification of Figure 8. Decreased tissue density, widened interfascicular spaces, large primitive-appearing fibroblasts, dearth of mature fibrocytes, reticular type of connective tissue fiber, and the increased number of extravascular lymphocytes are characteristic of marked clubbing. × 300.



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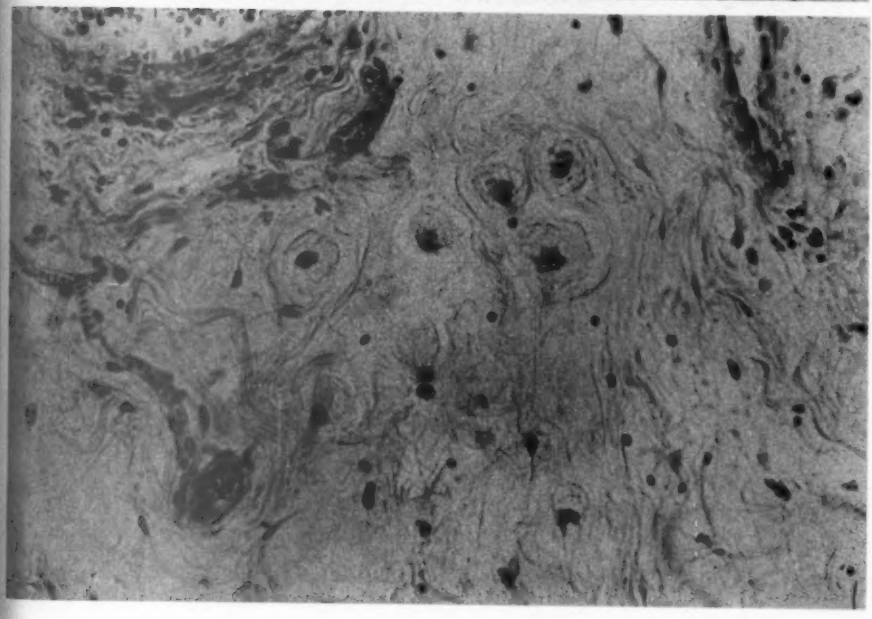

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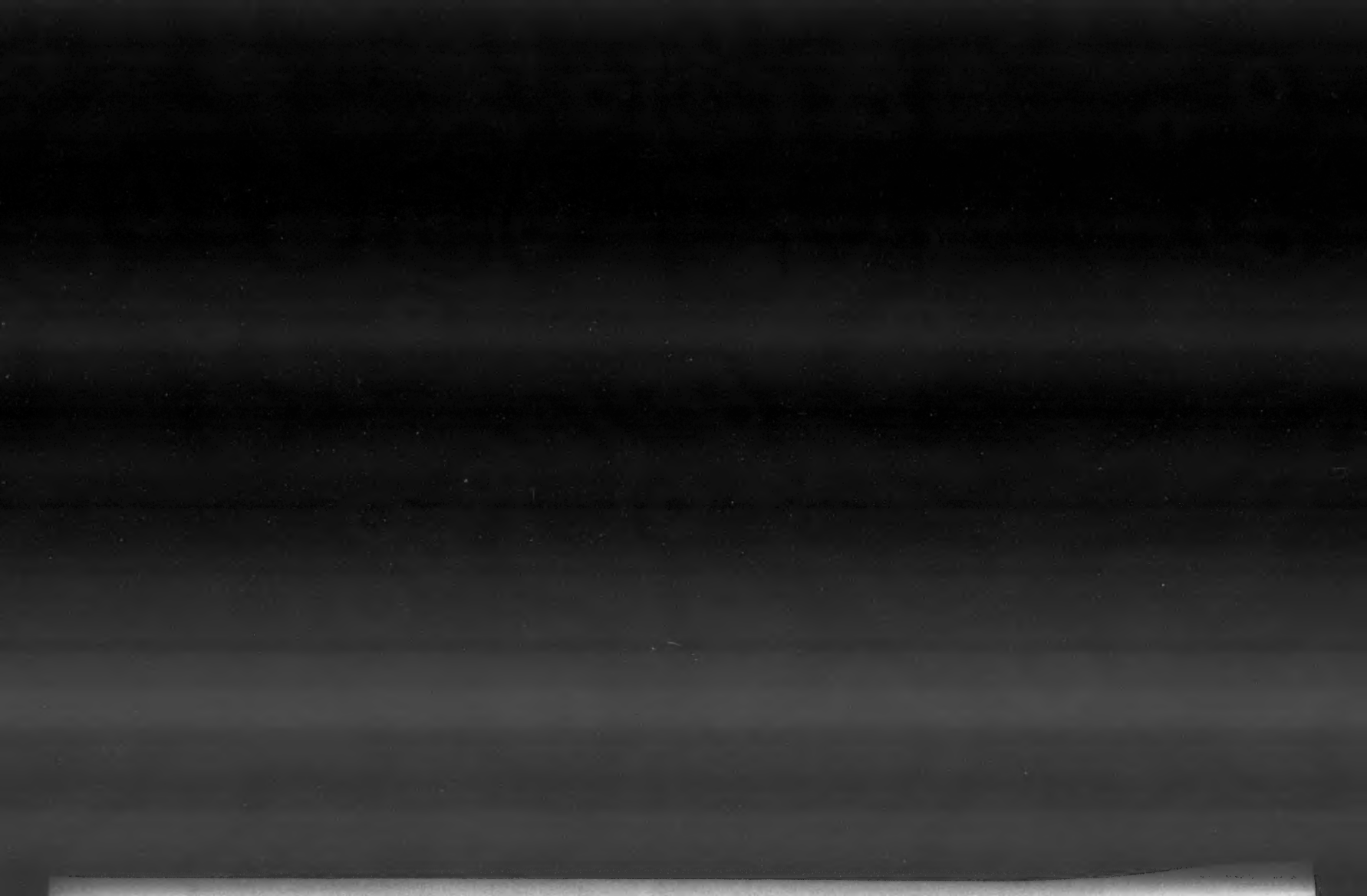
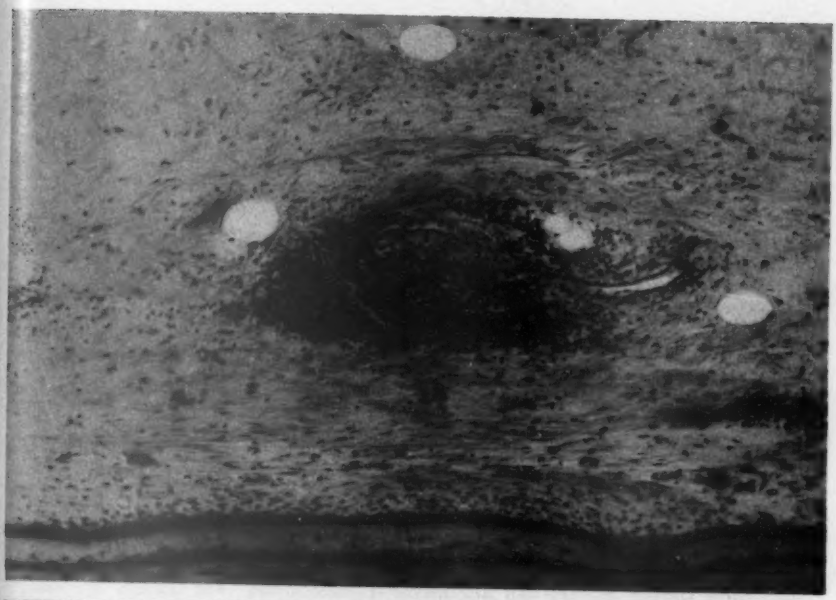
FIG. 11. Clubbed case 7. Periosteum of nailbed with bone in lower part of picture. Dense perivascular accumulation of lymphocytes is seen. × 120.

FIG. 12. Clubbed case 9, bone above. Loose tissue with widened interstitial spaces and vascularization noted in periosteum of palmar aspect of phalanx. Reference to Figure 6 will enable one to locate region in midsagittal section of thumb from which this highly magnified view is obtained. × 120.





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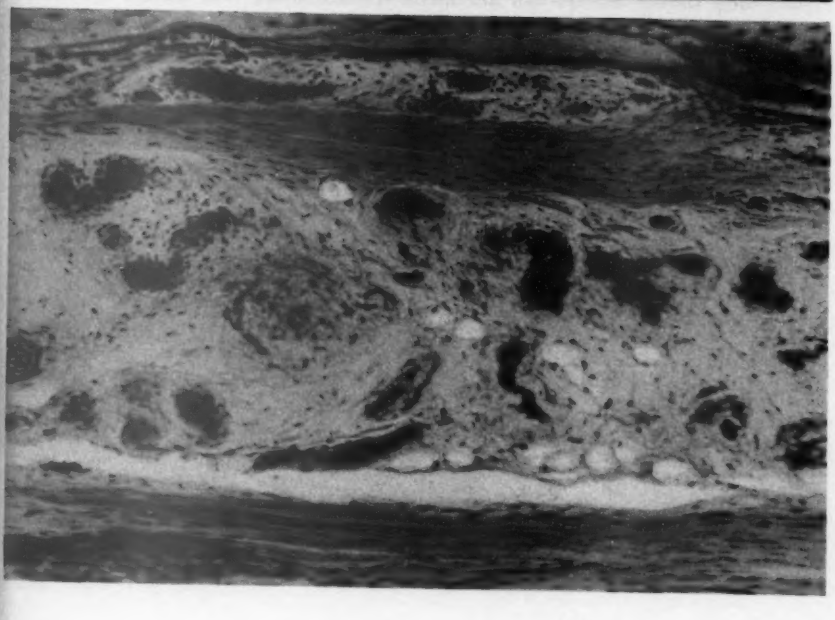

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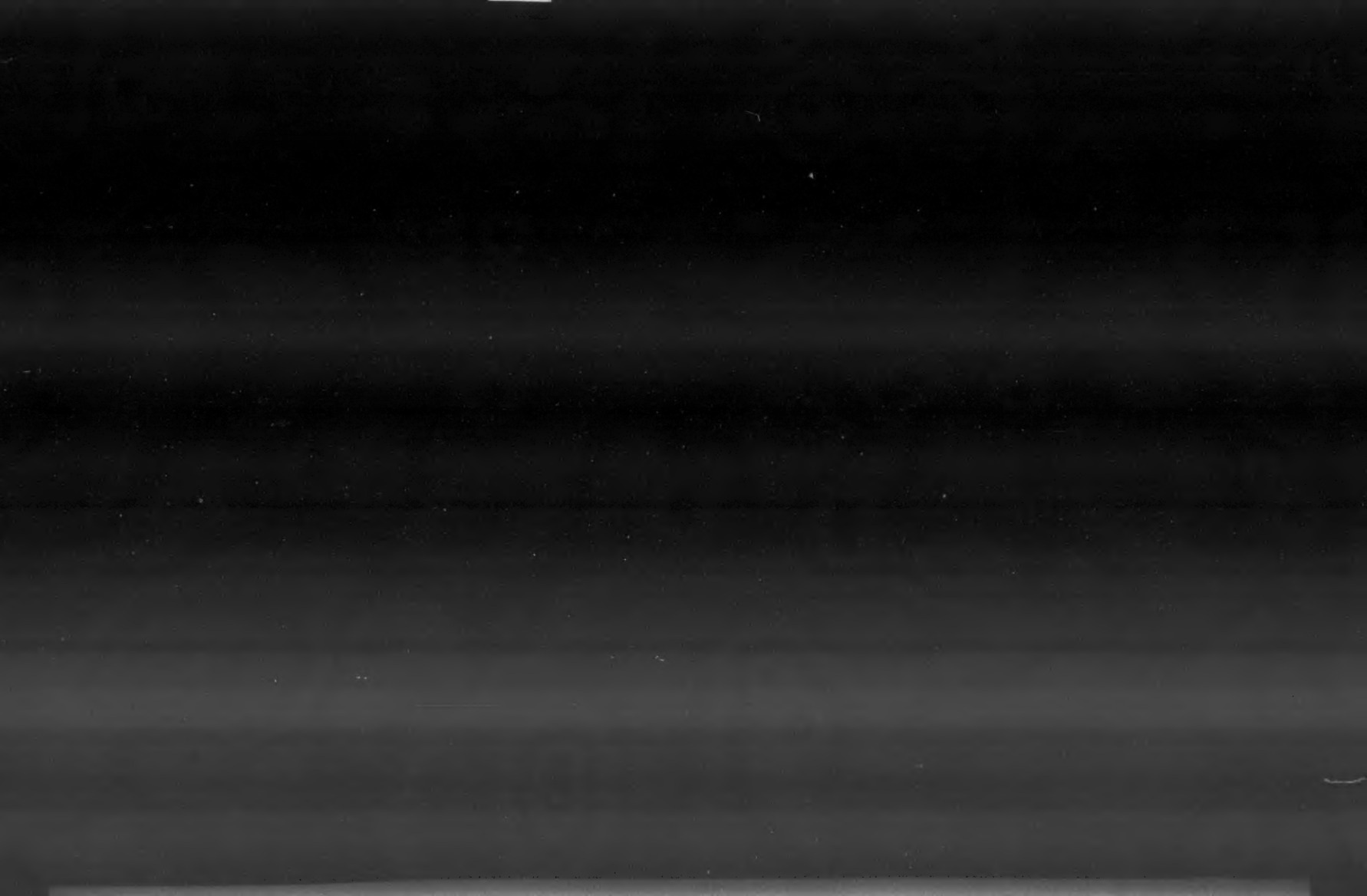
FIG. 13. Nonclubbed control, as in Figures 7 and 9. Glomus in distal portion of nailbed with bone inferiorly. Artery courses distally from left to right. Ramifications of artery are seen at right center. Glomus is composed of nutrient arterioles, collecting veins, nerve fibers, and a major artery which divides to form smaller arteries and arteriovenous anastomoses. The glomus in this illustration is the largest encountered in all the slides of the 29 control cases. × 72.

FIG. 14. Clubbed case 7. Region in middle portion of nailbed showing microscopic alteration in vascular pattern. An artery is on the right; the rest of the photograph is filled by whorls of vessels appearing to be arteriovenous anastomotic units. × 72.



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14

DIETARY PRODUCTION OF LIPOGRANULOMA IN RATS*

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During an investigation of some synthetic dietary fats, we noticed that rats which were fed acetylated monoglyceride of stearic acid developed focal granulomatous lesions in adipose tissue. The occurrence of these lesions clearly appeared to be related to the consumption of this acetylated compound.

The acetylation of natural fats and oils by interesterification, or of monoglycerides of long-chain fatty acids such as stearic or oleic acids, has resulted in the synthesis of fats with unusual properties. These substances have attracted interest as potentially edible fats and as food coatings. Of these acetoglycerides, two varieties, containing stearic acid and oleic acid respectively, were administered to experimental animals. These compounds will be referred to as acetostearin and aceto-olein. Five different preparations have been studied. They were derived from lard and from cottonseed oil by acetylation and fractionation. Inasmuch as all of the preparations of acetostearin produced similar effects, which differed from those evoked by the aceto-oleins, only the two major varieties of acetoglycerides will be distinguished here.

An initial long-term study included examination of 182 rats of both sexes. The acetoglycerides were added in 5, 10, and 20 per cent proportions to an adequate basal diet which was modified so that the protein content remained 17.6 per cent and the caloric value per 100 gm. remained approximately the same. The 20 per cent acetoglyceride diets were continued for 400 days and the others were maintained for 600 to 700 days.

The animals receiving the modified diets ate well, and their survival was comparable to that of control animals. While the average body weight of those which had consumed acetoglycerides was slightly less in most of the groups than that of controls, this difference was less than 15 per cent in all but one group.[1]

Adipose tissue lesions were first recognized in the small amounts of fatty tissue surrounding the abdominal organs which were subjected to histologic examination. Usually they were found adjacent to the

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stomach, intestine, pancreas or gonads. The incidence of these lesions in single sections of the abdominal organs is shown in Table I.

While Table I does not indicate the full incidence of lesions, it shows a clear relationship between the amount of acetostearin consumed and the prominence of lesions. In the single animal which developed lesions following aceto-olein feeding, the alteration of the fatty tissue was not pronounced. Further histologic study of additional tissue preserved as stock revealed lesions in every animal which received a diet of 20 per cent acetostearin, and indicated the virtual absence of lesions in animals which received aceto-olein. Lesions in the animals fed 10 per cent or 5 per cent acetostearin were less numerous and sometimes less well defined than in those receiving 20 per cent. There was, however, much variety in the extent of the process in different animals, and several given the 20 per cent diet presented fewer lesions than did certain of those fed the 10 per cent diet.

TABLE I

Incidence of Granulomatous Lesions in Single Sections of Fatty Tissue Attached to Abdominal Organs

Diet	% Synthetic fat in diet	Number of animals	Number with lesions
Acetostearin	20	48	45
Acetostearin	10	30	11
Acetostearin	5	29	5
Aceto-olein	20	26	1
Aceto-olein	10	15	0
Aceto-olein	5	21	0
Control	—	23	0

Other experiments with acetostearin feeding have demonstrated that lesions may appear as early as 30 days after the institution of an altered diet.

The lesions were focal, usually centered about one or several round spaces approximately the size of fat cells (Fig. 1). These spaces were bordered by large macrophages (Fig. 2), the cytoplasm of which was granular, eosinophilic, and did not contain stainable lipid. It frequently merged with similar eosinophilic material in a central space. This substance was commonly distorted by clefts suggesting sites of crystal deposit (Fig. 3); these corresponded to long, optically active crystals demonstrable in frozen sections. Occasional associated macrophages had foamy, lipid-containing cytoplasm. Frequent multinucleated giant cells had the appearance of fused macrophages. Neighboring fat cells often appeared entirely normal.

There was a mild infiltration with lymphoid cells about some of the lesions but many exhibited little more than the macrophage response. A few fibroblasts bordered the lesions, but these were not numerous. A few strands of material with the staining properties of collagen were deposited irregularly in some lesions, but none of the nodules had been converted to scars even after 700 days.

The distribution of the fatty tissue changes was not uniform. In general, the abdominal fat, which in the rat represents the principal fat depot, contained the greatest number of alterations. Lesions were found, however, in the mediastinal fat, the subcutaneous fat, and the deep fat of the neck. The sex of the animals did not influence the development of the pathologic process appreciably.

To test the capacity of other dietary fats to produce similar changes, diets containing butter or tallow in concentrations up to 50 per cent were fed to rats, but in these no adipose tissue lesions developed.

Since it has been observed[1] that acetostearin increases the requirement of the rat for Vitamin E, and that the decreased fertility of experimental animals receiving this substance can be counteracted by a Vitamin E supplement in the diet, a study was made of adipose tissue from rats which had received acetostearin and an α-tocopherol supplement of 14 mg. per animal per week. This did not prevent the development of the adipose tissue lesions.

Discussion

These granulomatous lesions of fatty tissue suggested a foreign body reaction, and the presence of fatty crystals, related to giant cells of the foreign body type, suggested the possibility that the crystals might represent the provocative foreign agent. The lesions in the experimental rats were reminiscent of human infantile fat necrosis, often called *sclerema adiposum neonatorum*, where a similar foreign body response around crystals is seen (Fig. 4). The postulation has been made repeatedly with respect to this condition that the cause might be related to a distinctive composition of infantile subcutaneous fat, in which the relative amount of higher melting point fats (stearates and palmitates) is said to be greater than in adults.[2] Siwe[3] produced granulomatous lesions in adipose tissue of guinea pigs by injecting palmitic acid or its esters. Reported instances of lipogranuloma appearing in children after refrigeration of the body prior to cardiac surgery[4,5] suggest that the physical state of the fat in the adipose tissue may be a causal factor in the production of adipose tissue injury.

Since it has long been known that the nature of the dietary fat influences the composition of the depot fat of the body, and because of the relatively high melting point of the acetostearins used in this study (up to 57°C), we suspected that the feeding of synthetic fat might influence the melting point of the depot fat in the experimental animals. Some components of depot fat, even when present in relatively small amounts, may change the melting point appreciably.[6] A preliminary study of the melting properties of the adipose tissue fat

from different groups of our experimental animals failed to identify sharply defined melting points of untreated fat samples. When fat was removed from the adipose tissue with ether, however, the material obtained from the animals which had received acetostearin, crystallized at a higher temperature, and crystals appeared more rapidly at lower temperatures, than was observed in fat from the control animals or from those which had received aceto-olein or large supplements of butter or tallow. Only the fat from acetostearin rats crystallized at 37°C.

Although these observations are consistent with the hypothesis that the lipogranulomatous lesion may result from an injurious effect related to the physical state of the fat in the adipose tissue, the possibility of a more specific chemical effect, produced by an abnormal lipid directly or indirectly related to acetostearin, has not been excluded. Many long-chain fatty acids may produce granulomatous reactions when introduced into tissues, and some lesions produced in this way have been similar to those described here.[7] It is possible that by modification of the local fat metabolism, some substance other than acetostearin, and different from the normal fatty components, may accumulate to cause injury. This concept with respect to the production of other forms of lipogranuloma has been discussed by Smetana and Bernhard.[8]

Although the idea of altered chemical composition of adipose tissue as a cause of lipogranuloma is not new, the possibility that such changes might relate to the composition of the diet has not received much attention. It is unlikely that the conditions of our experiments will be duplicated in human nutrition, but the fact that lesions in fatty tissues have been produced experimentally in animals by modification of the diet calls attention to the possibility that dietary factors may initiate or perhaps modify reactions in human adipose tissue. Consideration of this possibility would be pertinent particularly in those diseases where the pathogenesis is not clear, such as sclerosing lipogranuloma, insulin atrophy, and some types of so-called nonsuppurative panniculitis.

Summary and Conclusion

Feeding of the acetylated monostearate of glycerine to rats produced a focal granulomatous reaction in adipose tissue. It is suggested that the influence of dietary constituents upon the character of fatty tissue is a possible factor in human disease.

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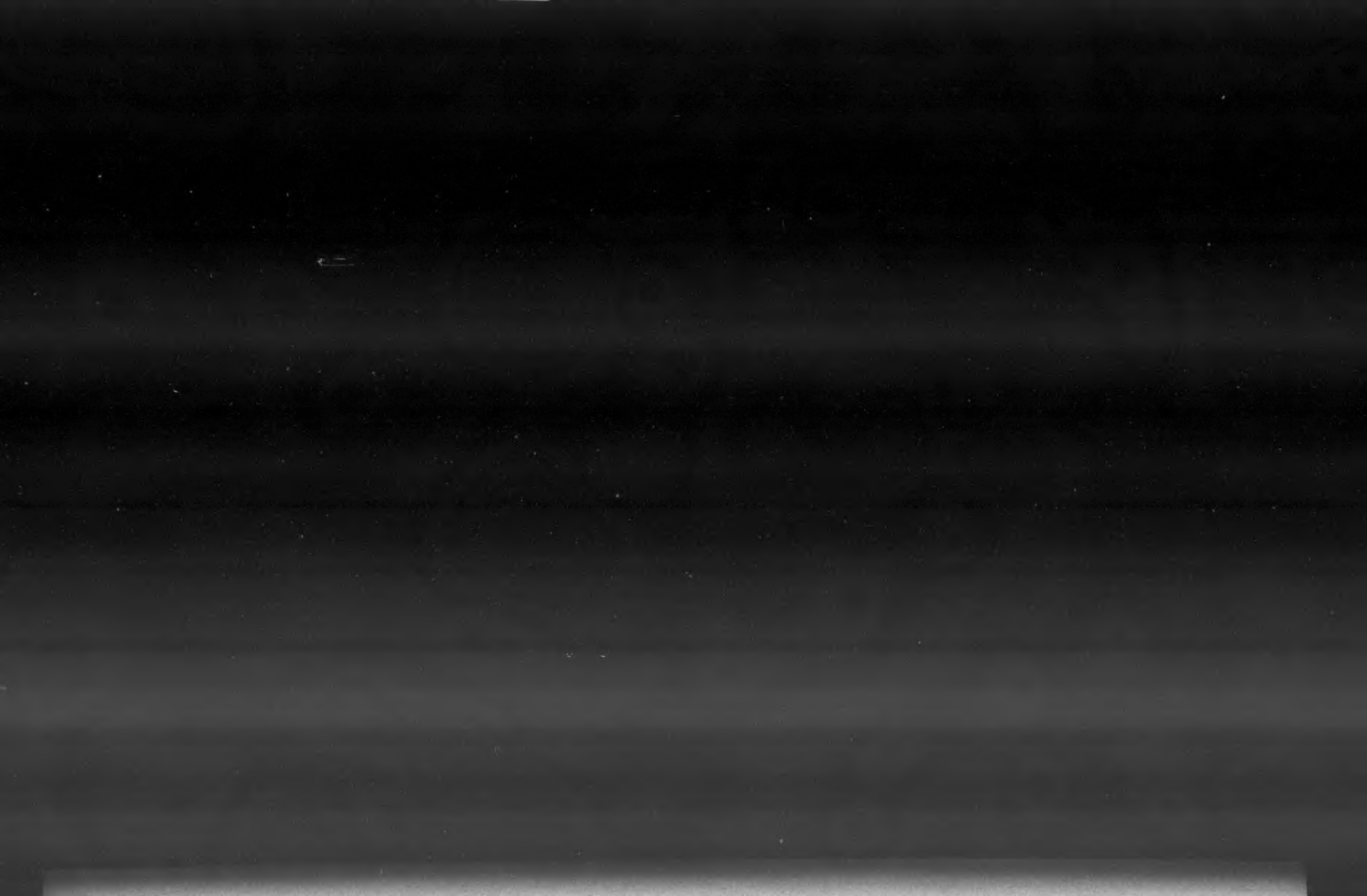
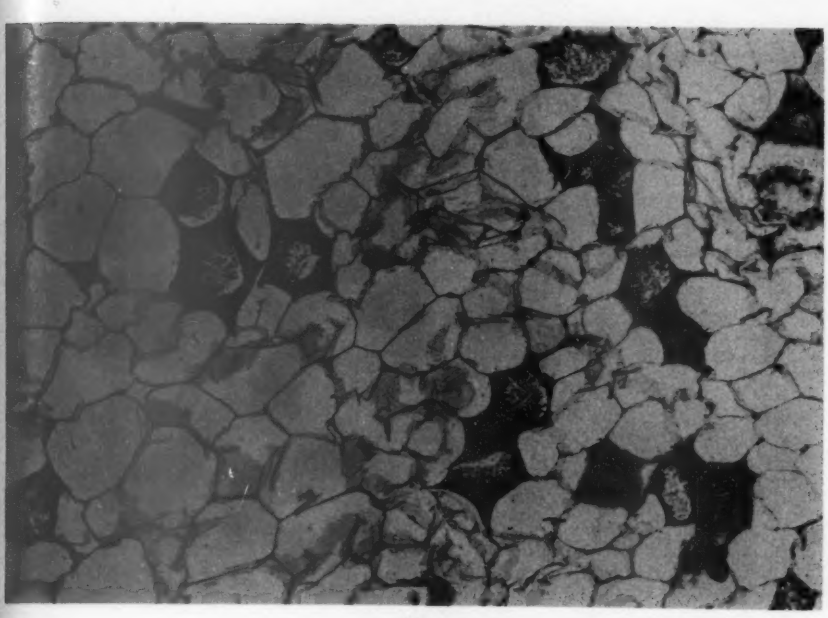
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Abdominal adipose tissue from a rat which was fed a diet containing 20 per cent acetostearin for 400 days. Focal lesions occupy spaces about the size of the surrounding distended fat cells. Hematoxylin and eosin stain. × 112.

FIG. 2. High power view of a conglomerate lesion, showing rings of large macrophages surrounding central acellular zones. Several multinucleated cells are present. Hematoxylin and eosin stain. × 440.





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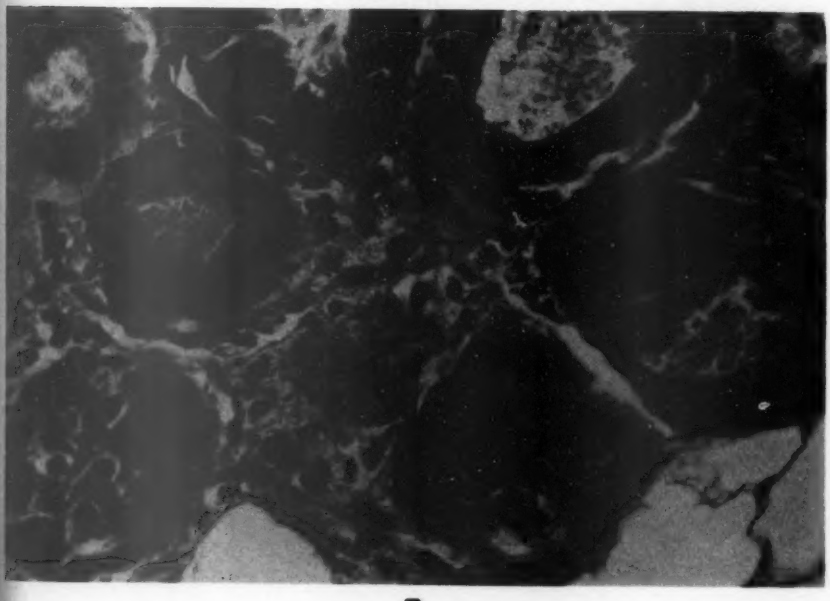

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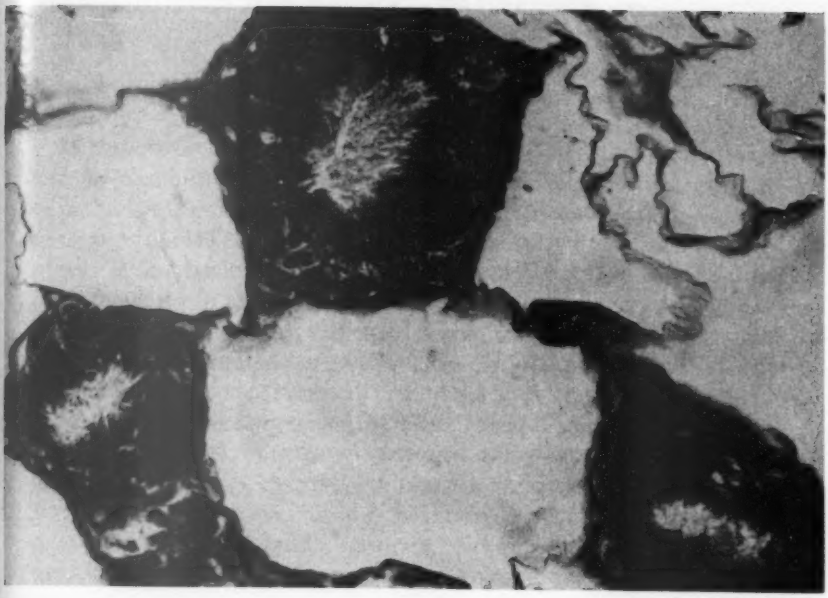
FIG. 3. Three isolated granulomatous foci are separated by normal distended fat cells. Fine clefts toward the center of the nodules correspond to crystals in frozen sections. Hematoxylin and eosin stain. × 440.

FIG. 4. A lesion of similar structure from the subcutaneous tissue of a child with *sclerema adiposum neonatorum*. Hematoxylin and eosin stain. × 440.



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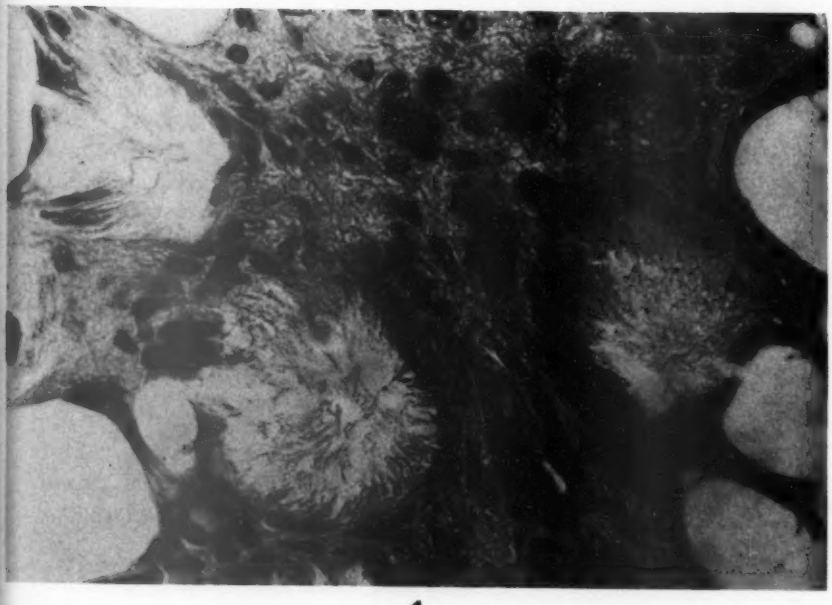

4

LEIOMYOMETAPLASTS, PARTICLES OF UNKNOWN SIGNIFICANCE IN SMOOTH MUSCLE CELLS*

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In the course of microscopic examination of hematoxylin and eosin stained tissue sections of a 1-year-old female bloodhound, particles of unknown significance were observed within cytoplasmic vacuoles of hypertrophied duodenal smooth muscle cells near the myenteric plexus of Auerbach (Fig. 1). These intracytoplasmic granules occurred most regularly in the *tunica muscularis interna* in aggregates measuring 20 to 160 μ in length and 5 to 18 μ in diameter. Each vacuolated cell contained hundreds of individually discrete particles of different sizes and shapes, approximately 1.2 to 1.8 μ in diameter, with deeply basophilic centers surrounded by clear halos. The nuclei of many of the hypertrophied, vacuolated smooth muscle cells were surrounded by these particles. Larger vacuoles, which appeared to have been formed by the merging of two or more smaller ones, occasionally contained several basophilic particles. No inflammatory response was associated with these lesions. Similar lesions were not recognized in any other sections examined, including those from practically all organs and tissues.

The immediate cause of death of this dog was pneumonia of a gangrenous type induced by the aspiration of food or other ingesta. Colonies of bacteria were observed in the lung, and thrombi containing similar bacteria were evident in the sections of tonsils and periorbital tissue. Acidophilic cytoplasmic and nuclear inclusions in the reticuloendothelial cells of the lymph nodes and in the gastric glandular epithelium were compatible with those of canine distemper.

The aggregates of particles within hypertrophied smooth muscle cells of the duodenum were thought, at first, to resemble the pseudocysts of *Toxoplasma gondii*. However, their restricted distribution and their somewhat smaller size raised serious doubt as to this possibility. An effort was made to identify the particles, and this report deals with the results of this study.

MATERIALS

Microscopic sections from dogs and cats submitted to the Armed Forces Institute of Pathology over a period of 6 months (September 1956 to April 1957) were screened for the presence of particles similar

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to those described. They were demonstrated in sections of formalin-fixed tissues from 3 additional animals.

Case 2. A 12-year-old castrated male domestic cat. AFIP diagnoses: (a) argentaffin carcinoma (carcinoid), large intestine, with metastasis to a lymph node; (b) acute enteritis of the small and large intestines; (c) subacute sclerosing nephropathy; (d) acute pancreatitis; and (e) acute focal hepatitis with ceroid deposits. Acidophilic and basophilic particles within cytoplasmic vacuoles of hypertrophied smooth muscle cells were present throughout the *tunica muscularis* of the duodenum and urinary bladder and in the trabeculae of the spleen (Fig. 2). They were not observed in lungs, myocardium, lymph node, liver, pancreas, stomach, or colon.

Case 3. A 12-year-old female cocker spaniel. AFIP diagnosis: carotid body tumor with multiple metastases to the lungs. Tissue sections of the larynx, trachea, lungs, mediastinal and mesenteric lymph nodes, myocardium, spleen, liver, pancreas, esophagus, duodenum, and kidneys were available for examination. Basophilic particles within cytoplasmic vacuoles of hypertrophied smooth muscle cells were present only in the *tunica muscularis* of the duodenum in the vicinity of the myenteric plexus of Auerbach (Fig. 3).

Case 4. A 4-year-old female golden retriever. From study of the tissues submitted to the AFIP, the cause of death could not be ascertained, for no significant lesions, except acute, generalized, passive hyperemia, were recognized. Tissue sections of the lungs, spleen, liver, duodenum, pyloric sphincter, kidneys, adrenals, pancreas, and myocardium were available for examination. Basophilic particles within cytoplasmic vacuoles of hypertrophied smooth muscle cells were present only in the *tunica muscularis interna* of the duodenum in the vicinity of the myenteric plexus of Auerbach (Fig. 4).

Methods

Serial sections, 5 to 6 μ in thickness, were prepared from the paraffin-embedded tissues which contained the particles in question. In order to ascertain that particles were present, each section was examined by phase microscopy before it was stained. All sections were stained by various techniques and subjected to histochemical tests.

One section of each tissue selected from a given case was deparaffined and examined microscopically, by means of conventional illumination. These sections were then mounted in glycerol and examined by polariscopy and fluorescent microscopy.

Deparaffined serial sections of the selected tissues from each case were stained by the following techniques:

1. For the demonstration of cell structure: Harris's hematoxylin and aqueous eosin,[1] Mallory's phosphotungstic acid hematoxylin,[2] Bodian's copper protargol,[3] Gomori-Burtner methenamine silver,[3] and toluidine blue metachromasia stain.[4]

2. For the demonstration of bacteria, fungi and protozoa: Jenner's Giemsa,[1] Brown and Brenn stain for gram-positive and gram-negative organisms,[1] Gridley's fungus stain,[5] and Ziehl-Neelsen acid-fast stain.[1]

3. For the demonstration of minerals: Von Kossa's silver nitrate,[3] Langeron's alizarin red S,[3] Gomori's iron reaction.[2]

4. For the demonstration of fats and lipids: Phosphomolybdic acid,[6] osmium tetroxide,[1] oil red O for 2 and 24 hours,[3] oil red O for 24 hours followed by 15 minutes in acetone,[4] phenylhydrazine-Schiff reaction.[4]

5. For the demonstration of mucin and mucopolysaccharides: Johnson's modified Alcian blue,[4] Mayer's mucicarmine.[1]

Three duplicate sets of deparaffined sections from each case were treated by the periodic acid-Schiff leucofuchsin reaction.[7] One set was stained by the phosphomolybdic acid technique[6] after oxidation with periodic acid and before immersion in Schiff reagent. On the second set the periodic acid-Schiff leucofuchsin reaction was performed as directed[7] and counterstained with Harris's hematoxylin, and the third set was counterstained by the phosphomolybdic acid technique.

Additional replicate sections were treated by the following procedures and subsequently subjected to the periodic acid-Schiff leucofuchsin reaction:

1. Acetylation for 24 hours with an anhydrous pyridine control; acetylation for 24 hours followed by de-acetylation for 12 hours;[3] and acetylation for 24 hours followed by de-acetylation for 12 hours and staining with phosphomolybdic acid after the periodic acid-Schiff leucofuchsin reaction.
2. Bromination for 24 hours with a carbon tetrachloride control.[3]
3. Extraction with hot methanol-chloroform 1:1 for 24 hours.
4. Digestion with human saliva for 30 minutes.

Results

Particles in cytoplasmic vacuoles of hypertrophied smooth muscle cells (Figs. 1 to 4) were present in all sections subjected to the various staining techniques and histochemical tests as determined by phase microscopy. In unstained deparaffined sections the particles had little

intrinsic color, being the same cream color as the adjacent muscularis. Deparaffined tissue sections of several normal dogs were examined and their intestinal muscularis was the same color. The particles were not birefringent and yielded a dim yellow fluorescence.

In the tissue sections from Cases 1, 3 and 4, stained with Harris's hematoxylin and aqueous eosin, the particles exhibited a deep basophilic center and were surrounded by a clear halo (Fig. 2). Those in the tissue sections from Case 2 varied in their reaction to the stain. Most of them were acidophilic, a few basophilic, and a few remained unstained. Regardless of the staining reaction, the structure of the particles was the same as in the other 3 cases (Fig. 3). The results obtained with all the other techniques were identical for the 4 cases. Therefore, they will be described collectively.

The particles remained unstained when sections were stained with Mallory's phosphotungstic acid hematoxylin or Bodian's copper protargol. They were covered by a light deposition of silver in sections treated by the Gomori-Burtner methenamine silver technique, but the internal structure of the individual particles was not discernible. Metachromasia was not demonstrated with toluidine blue stain. When stained by each of the techniques employed for the determination of bacteria, fungi, protozoa, and minerals they remained uncolored. The reaction of the particles to Johnson's modified Alcian blue and Mayer's mucicarmine techniques was also consistently negative.

With the phosphomolybdic acid technique, the periphery of each particle became bluish green and its center took a lighter stain or remained unstained. The particles did not reduce osmium tetroxide within 24 hours, and the phenylhydrazine-Schiff reaction was negative. In sections immersed in oil red O for two hours, they exhibited a faint brown color. When left in this stain for 24 hours, the particles took on a light brown color which was readily removed by immersion in acetone for 15 minutes.

In sections subjected to the periodic acid-Schiff-leucofuchsin reaction the particles were positive. When counterstained with Harris's hematoxylin their centers stained intensely and their halos faintly (Fig. 4). Counterstain by the phosphomolybdic acid technique caused the center of each particle to become deep purple and the periphery light blue. When the same technique was interposed between the oxidation and the Schiff staining procedures, the entire particle assumed a deep bluish purple color. After acetylation the particles failed to give a positive periodic acid-Schiff leucofuchsin reaction. After de-acetylation, the reaction was positive and similar to that which has been described for preparations counterstained with either

Harris's hematoxylin or the phosphomolybdic acid technique. In control sections exposed to anhydrous pyridine this reaction was strongly positive. Subsequent to bromination, the particles gave only a faint positive periodic acid-Schiff leucofuchsin reaction. In control sections exposed to carbon tetrachloride, a strong positive reaction was obtained. Positive reactions were also seen after treatment with hot methanol-chloroform or human saliva. In all 3 instances the structure of the particles was adequately demonstrated.

Discussion

These results indicate that the particle in question, in the cases studied, is a definite morphologic entity. It is probably composed of an oxidized or polymerized unsaturated lipid associated with phosphorus surrounding a center containing vicinal glycolic groups. It can be defined as a nonbirefringent particle, 1.2 to 1.8 μ in diameter, occurring in intracytoplasmic vacuoles within hypertrophied smooth muscle cells. It exhibits little intrinsic color and yields a dim yellow fluorescence in paraffin-embedded sections. It is usually basophilic in hematoxylin and eosin-stained preparations, and does not take up the usual stains employed for bacteria, fungi, or protozoa. It is positive to the periodic acid-Schiff reaction; it is unaffected by digestion, decreased in intensity by bromination, blocked by acetylation and restored by de-acetylation. It is insoluble in the usual fat solvents, but stains with oil red O and phosphomolybdic acid in paraffin-embedded sections. It reduces methenamine silver, but not osmium tetroxide and potassium ferrocyanide. When stained with mucicarmine or Alcian blue, it gives a negative reaction. It is suggested that these particles be designated by the term "leiomyometaplast," a descriptive term which characterizes them as particles of lifeless matter, or inclusions within the cytoplasm of smooth muscle cells.

The reason for the predominantly acidophilic staining reaction of the leiomyometaplasts observed in Case 2 is not understood. The structure of the acidophilic particles was identical to that of similar particles in the other cases. Their responses to staining techniques and histochemical procedures were otherwise identical to those obtained with the particles in Cases 1, 3, and 4. These observations leave little doubt that the particles in all cases are essentially the same.

Although it is believed that the leiomyometaplasts are associated with a specific lesion of smooth muscle, in the cases observed there was no disease with which they could be identified. To date they have been observed only in dogs and cats ranging in age from 1 to

12 years. There are several intracellular parasites and pigments in animals which are somewhat similar and from which these particles must be distinguished. The leiomyometaplasts can be differentiated readily from pseudocysts containing toxoplasma. A pseudocyst does not attain the proportions of an aggregate of metaplastic granules, and the individual toxoplasma is larger than a single particle. Pseudocysts arise within the host cell by invasion and subsequent multiplication of toxoplasma.[8] As the pseudocyst develops, the cell becomes distended and the nucleus obscured or degenerated, leaving a mass of toxoplasma surrounded by a limiting membrane formed from the wall of the host cell.[8] In cells containing leiomyometaplasts, the nucleus is not obscured, and although the cell is hypertrophied, portions of the cytoplasm are still apparent. Normal-appearing nuclei are frequently surrounded by numerous particles. Because these metaplastic granules do not stain by techniques commonly used for the demonstration of bacteria, fungi and protozoa, it is believed that they are not parasitic organisms.

The mucinous granules of basophilic degeneration of the myocardium resemble the leiomyometaplasts in some respects. The intracellular material in basophilic degeneration has been described as a mucoprotein or acid mucopolysaccharide[9-12] which exhibits a distinct metachromasia with toluidine blue.[9] In the cases recorded in this study, granules were not recognized in the myocardium. Leiomyometaplasts were observed only within vacuolated, hypertrophied smooth muscle cells, and they yielded negative reactions when stained for mucin or mucopolysaccharide. They did not exhibit metachromasia when stained with toluidine blue. These divergent reactions suggest that they and the mucinous granules of basophilic degeneration of the myocardium are separate entities.

In establishing the identity of the leiomyometaplasts, they must be differentiated from the various lipofuscin pigments.[8] They are similar in that they are negative to iron stains and positive to the periodic acid-Schiff stain. The latter reaction is blocked by acetylation, but not by bromination or digestion with saliva. In paraffin-embedded sections they stain with oil soluble dyes, with the exception of von Recklinghausen's hemofuscin.[8]

In brown atrophy of the heart in animals,[13] cardiac lipofuscin granules are observed within myocardial fibers. Of the various lipofuscins, these granules bear the closest morphologic resemblance to leiomyometaplasts. They are pigmented and appear as dark brown particles in unstained sections and in sections stained with hematoxylin and eosin and oil red O. They are laid down at either pole of the nucleus

of myocardial cells[12,13] and are occasionally acid-fast.[3] Leiomyometaplasts, on the other hand, exhibit little intrinsic color in unstained sections and are usually basophilic but occasionally acidophilic when stained with hematoxylin and eosin. They occur within cytoplasmic vacuoles of hypertrophied smooth muscle cells, not in myocardial fibers, and their distribution is not predominantly polar. When treated with acid-fast techniques they remain unstained.

In general, the other lipofuscin pigments are yellowish brown in unstained deparaffined preparations and yellowish brown or basophilic in sections stained with hematoxylin and eosin, whereas the granule described exhibits little intrinsic color and contrasts with many of the lipofuscins which are acid-fast or partially so. Osmium tetroxide is reduced by the lipofuscins[2] but not by leiomyometaplasts. Those lipofuscins which are fluorescent in paraffin-embedded sections yield a golden to red brown fluorescence[2] as compared to a dim yellow fluorescence. Under most conditions the lipofuscins are not readily de-acteylated[4] whereas the leiomyometaplast is. For these reasons and because of the characteristic occurrence of leiomyometaplasts within cytoplasmic vacuoles, they are not identifiable as lipofuscin or lipofuscin-like pigment.

The staining characteristics of the leiomyometaplasts and their lack of any significant intrinsic color supply no evidence that they are endogenous or exogenous pigments. The outer portions of the particles exhibit some of the characteristics of carbonyl lipoids and plasmalogens[2] in that they are stained by oil soluble dyes and the phosphomolybdic acid technique. They differ in that they are relatively insoluble in fat solvents, are not birefringent, and produce a negative phenylhydrazine-Schiff reaction.

Leiomyometaplasts are thus distinct morphologic entities which have been demonstrated within cytoplasmic vacuoles of hypertrophied smooth muscle cells of the dog and cat. They exhibit characteristic staining qualities, histochemical reactions and physical properties by which they may be identified. Their significance and origin are not known. Further investigations are being conducted in an effort to ascertain their significance.

Summary

Particles of unknown significance were observed within intracytoplasmic vacuoles of hypertrophied smooth muscle cells of the duodenum in 3 dogs, and of the duodenum, spleen and bladder of a cat. Their characteristics were determined by a variety of staining techniques, histochemical tests and physical procedures and they were

believed to constitute a distinct morphologic entity. It is suggested that they be designated leiomyometaplasts, a descriptive term which characterizes them as particles of lifeless matter or inclusions within the cytoplasm of smooth muscle cells.

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LEGENDS FOR FIGURES

FIG. 1. Duodenum of dog (Case 1), Armed Forces Institute of Pathology Accession 738967. Basophilic leiomyometaplasts in cytoplasmic vacuoles in smooth muscle cells of the *tunica muscularis interna* near the myenteric plexus of Auerbach. Hematoxylin and eosin stain. × 500.

FIG. 2. Spleen of cat (Case 2), AFIP Accession 535352. Leiomyometaplasts in cytoplasmic vacuoles in smooth muscle cells of a splenic trabecula. Periodic acid-Schiff reaction. × 1,000.

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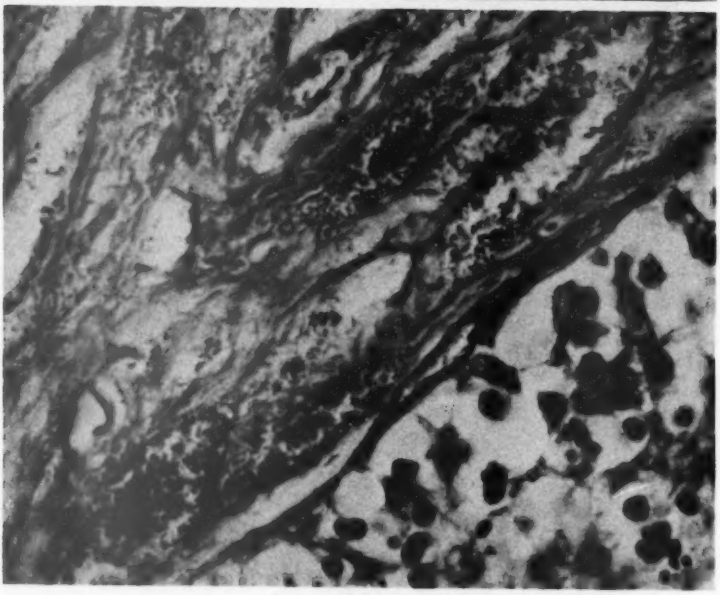

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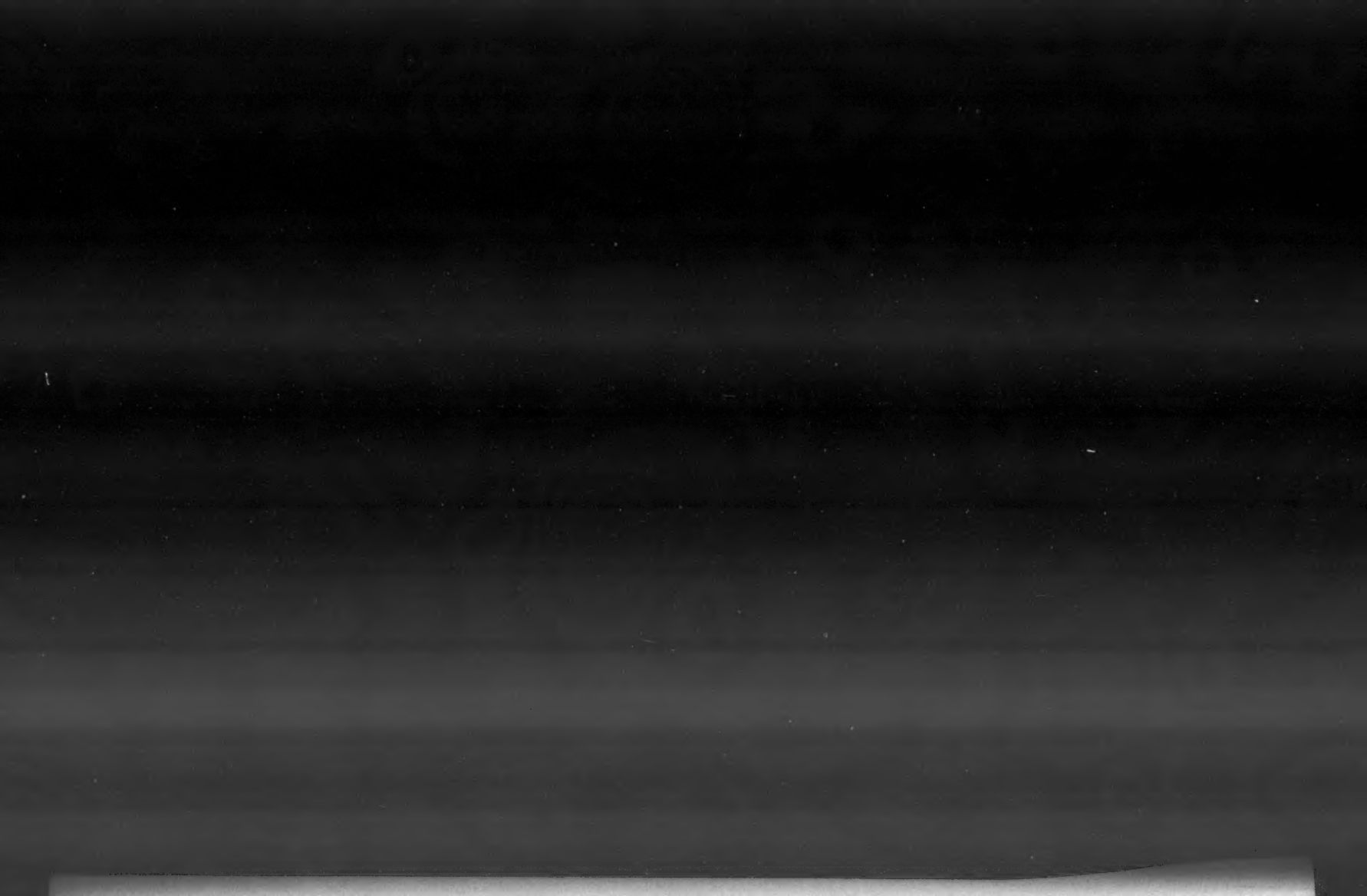
FIG. 3. Duodenum of dog (Case 3), AFIP Accession 768377. Leiomyometaplasts in cytoplasmic vacuoles of smooth muscle cells of the *tunica muscularis interna*. Periodic acid-Schiff reaction. × 1,000.

FIG. 4. Duodenum of dog (Case 4), AFIP Accession 784765. Leiomyometaplasts in cytoplasmic vacuoles of smooth muscle cells of the *tunica muscularis interna*. Periodic acid-Schiff reaction. × 2,100.



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4

GASTRIC OSTEODYSTROPHY*

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It is a well established fact that chloride deficiency can produce nephrocalcinosis both in man and in experimental animals. For example, in patients suffering from persistent vomiting or intense diarrhea, the loss of chloride through the gastrointestinal tract may lead to calcium deposition in the kidney.[1-4] In animal experiments, this type of nephrocalcinosis is most readily produced by ligation of the pylorus[3,5] or by maintenance on a chloride-deficient diet.[6] It is generally assumed that the development of hypochloremic alkalosis favors the precipitation of calcium salts, especially in the kidney where the production of an acid urine induces a sudden shift of the pH towards alkalinity.

In patients with peptic ulcer who have been treated for long periods of time with diets containing excessive amounts of milk and alkali, nephrocalcinosis is likewise common. In a few of these cases, the calcification in the kidney has been associated allegedly with radiologic evidence of excessive bone formation.[7] In a sense, such skeletal lesions would be the reverse of the so-called "renal osteodystrophy" which currently is interpreted as being due (directly or through the intermediary of the parathyroids) to acidosis, with consequent dissolution of bone salts.[8-10]

Although numerous studies deal with the bone lesions induced by renal acidosis, we were unable to find any data concerning skeletal alterations in response to hypochloremic alkalosis. It is the object of this communication to report upon experiments showing that extraordinary histologic changes do occur in the bones within two days after a surgical operation which completely prevents the reabsorption of gastric juice.

METHOD

Simple ligature of the pylorus is not suitable to insure the prolonged loss of gastric secretion in the rat since it usually results in death within the first 24 hours. After the pylorus is obstructed, a large amount of gastric juice accumulates in the stomach and is soon re-

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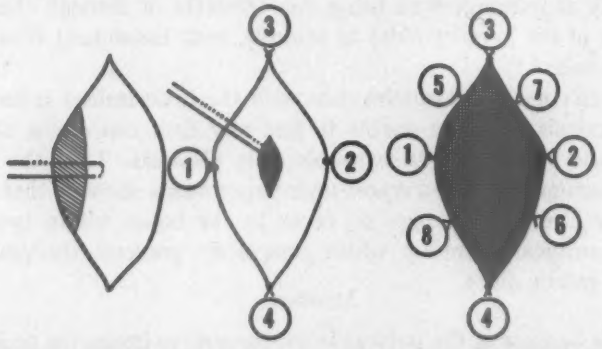
gurgitated into the esophagus. An acute hemorrhagic esophagitis is followed by perforation of the esophagus and flooding of the thoracic cavity with gastric juice.[11]

To prolong survival, an operation has been designed in which, following ligature of the pylorus, a gastric fistula is established. This permits the easy escape of gastric juice to the outside, thereby preventing regurgitation into the esophagus. The operation is performed as follows: After the lower thoracic and upper epigastric region is shaved, the rat is anesthetized with ether. The stomach is exposed through an incision 1.5 cm. in length, made in the midline just below the xiphoid process, through the skin and *linea alba*. The pyloric end of the stomach is now pulled through the wound, and a simple ligature is placed around the pylorus to obstruct the lumen. A sharp line of demarcation separates the pink ventriculus, lined by secreting gastric mucosa, from the whitish preventriculus with mucosa devoid of glandular cells. A single stitch, penetrating all layers of the gastric wall, is placed in the preventriculus near the borderline of the ventriculus, as indicated in Text-figure 1. The thread is then fastened to the abdominal wall on the right side of the rat, by means of a stitch that penetrates through peritoneum, muscles and skin, in that order. (No. 1 in the Text-figure.)



Text-figure 1. Schematic representation of the operative technique for the production of a gastric fistula in the rat. The numbers represent the order in which the stitches are to be placed.

A similar stitch (No. 2) is placed on the opposite side, fixing approximately 1.5 cm. of preventriculus mucosa between the lips of the wound. Next, similar stitches (Nos. 3 and 4) attach the preventriculus to the upper and lower tips of the incision. The wall of the preventriculus is incised near the middle of the operative field and 4 additional stitches (Nos. 5 to 8) are made as follows: the needle

is put into the gastric cavity and then thrust through mucosa, gastric wall, peritoneum, abdominal muscles and skin, in that order. Under these conditions, rats can survive for 48 hours or more after complete occlusion of the pylorus; esophageal perforations never occur.

Thirty female Sprague-Dawley rats, with an average body weight of 85 gm. (81 to 92 gm.), were subdivided into 3 equal groups. Group I served as untreated normal controls. In Group II the pylorus was ligated, and a gastric fistula was made according to the technique just described. In Group III the stomach was resected between ligatures placed on the pylorus and cardia. This last control was deemed necessary in order to ascertain the effect of a major gastric operation combined with the impossibility of food ingestion during the period of observation. Three animals of Group II died in the course of the second day after the operation. The survivors of all 3 groups were sacrificed 48 hours after initiation of the experiment, at which time several of the rats in Groups II and III were moribund.

Specimens of the ribs, femurs and tibias of all rats were fixed and simultaneously decalcified in SUSA solution, embedded in paraffin, and stained with hematoxylin and eosin. In addition, the kidneys were fixed in neutral formalin for the subsequent demonstration of calcium with von Kossa's silver nitrate technique.

Results

In the animals having gastric fistulas (Group II), pronounced histologic changes were uniformly detectable in all the bones examined. These lesions proved to be indistinguishable from the osteitis fibrosa which can be produced in the rat by acute administration of very high doses of parathyroid hormone or by total nephrectomy.[12] As a result of intense osteoclastic bone absorption, the primary bone spicules in the zone just below the growth cartilage discs had largely disappeared. In this region the myeloid tissue was almost completely replaced by a loose, edematous connective tissue that contained foci of necrosis and many large osteoclasts. On the other hand, in the rats whose stomachs had been completely removed (Group III), there was merely some osteoporosis—presumably due to stress and starvation—but there was no evidence of osteitis fibrosa (Fig. 2).

The sections stained with von Kossa's technique revealed varying degrees of nephrocalcinosis in the rats with gastric fistulas (Group II). This was characterized by the formation of calcified casts, particularly at the line of cortico-medullary junction in the kidney. No sign of nephrocalcinosis was noted in the two other groups.

Discussion

Our observations did not give us any information concerning the ultimate mechanism through which the deviation of gastric juice to the outside affects the skeleton. It is noteworthy, however, that the histologic character of the osseous changes produced by this operation was essentially the same as that of the bone lesions resulting from hyperparathyroidism or complete bilateral nephrectomy in the rat. It had been thought that, in the latter condition, skeletal changes were due to acidosis. Clinical observations suggested that chronic alkalosis tended to enhance the formation of calcified bone tissue.[7] Therefore, it was somewhat unexpected to note that loss of gastric juice, known to produce marked alkalosis, far from causing calcium retention in the bones, led to a dissolution of the calcified bone trabeculae—and a condition greatly resembling experimental "renal osteodystrophy." This was considered to represent a "gastric osteodystrophy."

Summary

A simple technique for the production of pyloric obstruction with a gastric fistula in the rat has been described. Following this operation a type of "gastric osteodystrophy" developed with histologic characteristics indistinguishable from those of the "renal osteodystrophy" appearing after complete nephrectomy or after various types of experimental renal damage. The condition was characterized by the absorption of trabecular bone, especially in the region just underneath growth cartilage plates, and the proliferation of connective tissue rich in osteoclasts. These changes were associated with nephrocalcinosis. Complete gastrectomy did not produce similar changes either in the skeleton or in the kidneys.

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[*Illustrations follow*]

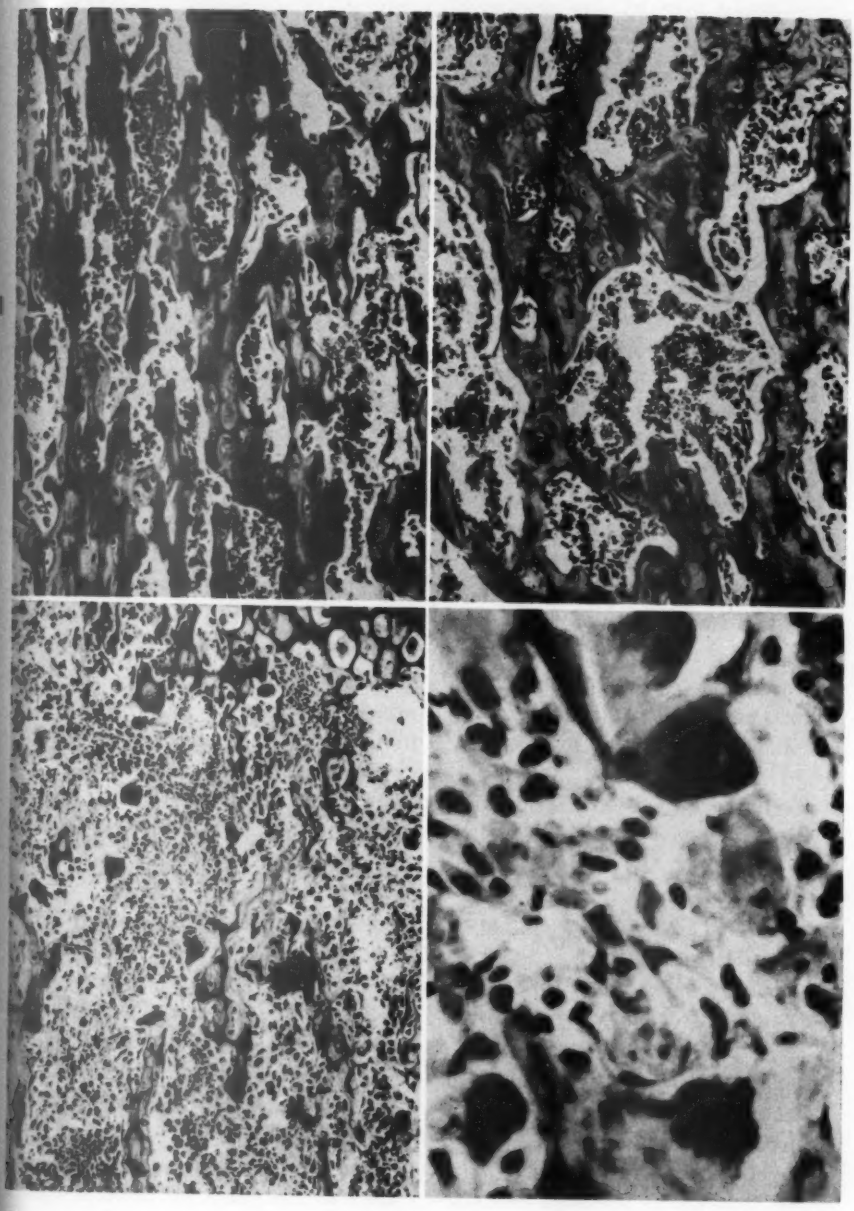
LEGENDS FOR FIGURES

FIG. 1. Normal primary bone spicules just underneath the growth cartilage plate of the proximal tibial epiphysis of a normal rat. Hematoxylin and eosin stain. × 150.

FIG. 2. Comparable bone region in a rat on which a complete gastrectomy was performed. There is slight osteoporotic loosening of the trabecular structure, without other abnormalities. Hematoxylin and eosin stain. × 150.

FIG. 3. Comparable bone region in a rat in which a gastric fistula was created. Most of the bone trabeculae have been dissolved, and the hemopoietic marrow is largely replaced by a loose, edematous connective tissue containing many unusually large osteoclasts. Hematoxylin and eosin stain. × 120.

FIG. 4. High magnification of a region from the preparation shown in Figure 3. × 500.



CHEMODECTOMAS OCCURRING CONCURRENTLY IN THE NECK (CAROTID BODY), TEMPORAL BONE (GLOMUS JUGULARE) AND RETROPERITONEUM

Report of a Case with Histochemical Observations*

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Rare, solitary carotid body tumors of the neck have long been recognized and their characteristic histologic features described.[1-3] More recently, multiple tumors of similar architecture, occurring in sites distant from the neck, have been studied. Rosenwasser[4] reported the first glomus jugulare tumor, and Kipkie,[5] the first concurrence of carotid body and glomus jugulare tumors. Cragg[6] recorded a case of concurrent tumors of the carotid body and both organs of Zuckerkandl, and Goodof and Lischer[7] described a case in which a carotid body tumor was associated with a microscopically similar neoplasm in the pancreas. Tumors of this type have now been reported from such sites as the middle ear, the nodose ganglion, the aortic and carotid bodies, the orbit,[8] the pancreas and the organs of Zuckerkandl. To avoid use of terminology suggesting sympathetic origin or specific sites of occurrence, the term "chemodectoma" used by Mulligan[9] seems preferable as a general term to include multicentric tumors microscopically resembling the carotid body tumor. Although this term leaves much to be desired, it does emphasize the physiologic role of the parent tissue without implying a specific site or embryologic origin. The present report describes a case in which four chemodectomas, including two in the retroperitoneal region were found at necropsy.

Case Report

In 1935 at 20 years of age, the patient was examined in the Massachusetts Eye and Ear Infirmary because of deafness in the left ear, hoarseness, and difficulty in breathing through the left nostril. Examination showed firm swelling of the lateral pharyngeal and nasopharyngeal walls extending to the choana. Three biopsy specimens revealed "chronic inflammation," and a fourth showed "cavernous hemangioma." In April, 1936, the left carotid artery was ligated at the level of the thyroid cartilage. Two days later the left pharyngeal wall was explored, and a highly vascular fibrous mass was excised. The patient remained well for 5 years and returned to the hospital

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because of a nontender swelling in the right side of the neck which had been present for one year. Roentgenograms showed marked right retropharyngeal and lateral pharyngeal swelling with displacement of the hyoid bone and larynx to the left. A lymph node removed from the right side of the neck above the carotid bifurcation, revealed "lymphoid hyperplasia." A short course of x-irradiation was given.

He re-entered the Massachusetts General Hospital in November, 1956, complaining of persistent hoarseness due to left vocal cord paralysis, a dry cough, deafness of the left ear, and moderate difficulty in swallowing solids. The left auditory canal contained a mass of foul-smelling granulation tissue. A firm mass, involving the left side of the palate, obstructed the eustachian tube, and a swelling of the right pharyngeal wall extended into the pyriform sinus, producing deviation of the larynx to the left. The base of the tongue was fixed on the left, and both vocal cords were immobile. A large, nontender, nonpulsatile, firm mass was present in the right side of the neck, extending above the mandible and protruding into the pharynx.

Roentgenographic examination of the skull disclosed destruction of the left middle fossa, the left petrous apex and part of the labyrinth. A barium swallow showed the valleculae and the pyriform sinus to be displaced to the left but not invaded.

Exploration of the right neck revealed, at the carotid bifurcation, a well encapsulated purplish tumor which bled profusely. For this reason, no attempt was made to remove it. Two days later, a mass of hemorrhagic soft blue tissue was removed from the left external auditory canal; microscopically this resembled a glomus jugulare tumor.

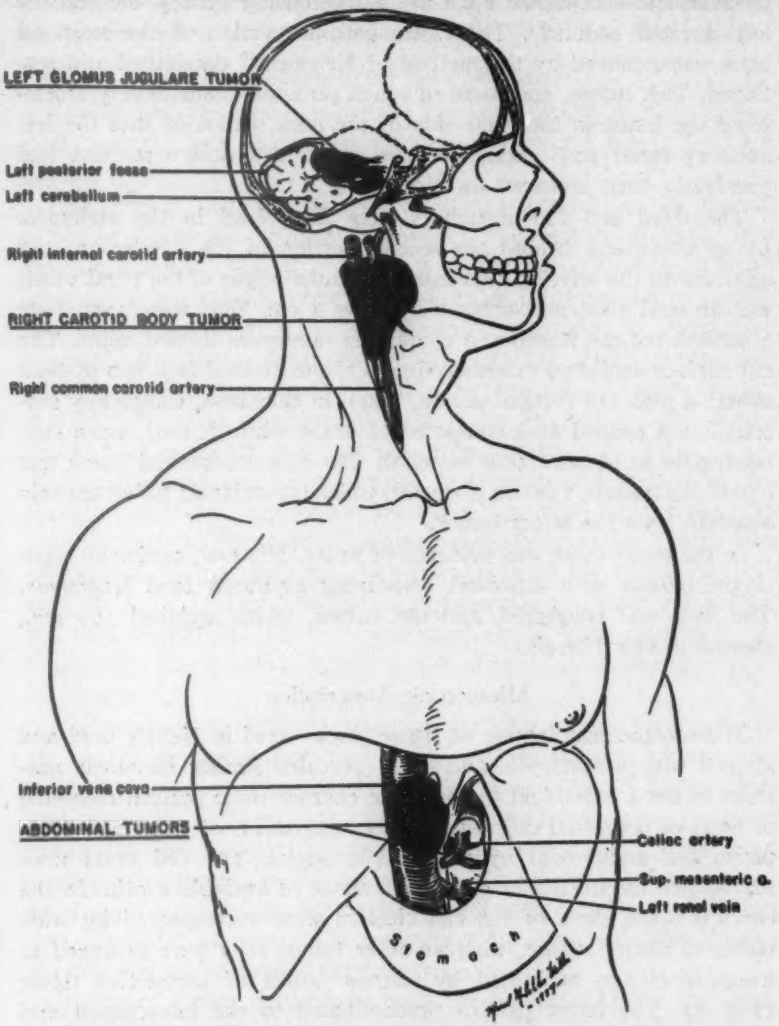
The patient had been examined several times during the preceding 3 years because of weakness and anemia. Examination of the blood showed 8.4 to 10.4 gm. of hemoglobin per hundred cc., normal differential blood counts and a few bizarre red cells in the smear. There was mild erythroid hyperplasia of the marrow. On one occasion, the urine urobilinogen was increased. A diagnosis of low-grade hemolytic anemia was made, and Meticorten therapy was begun. The patient died of bronchopneumonia in December, 1956.

Pathologic Observations

Four distinct tumors of similar gross and microscopic appearance were found at necropsy (Text-figure 1).

A tumor measuring 8 by 6.5 by 1.5 cm. was present in the right side of the neck and extended upward into the floor of the mouth and downward along the course of the common carotid artery. The carotid bifurcation and a segment of the common carotid artery were

embedded within the tumor. The cut surface of the mass revealed slightly lobulated, extremely tough, pink-tan homogeneous tissue (Fig.



Text-figure 1. Drawing indicating the sites of multicentric chemodectomas.

1), which was firmly adherent to the walls of the carotid arteries and to the subcutaneous tissue beneath the mandible. Multiple lymph nodes, including two large nodes from the left neck, were free of neoplasm. The left carotid artery, which had been ligated previously, was represented by a thin fibrous cord.

When the brain was removed, a large, smooth-surfaced, dull pink oval mass, measuring 6 cm. in diameter, projected from the left petrous pyramid and encroached upon the left cerebellar hemisphere and the left cerebral peduncle. The entire petrous portion of the temporal bone was removed by the method of Kelemen,[10] decalcified and sectioned. This tumor, composed of tough pink-tan tissue closely resembling the lesion in the right side of the neck, extended into the left auditory canal and represented the tissue from which sections had previously been procured for biopsy.

The third and fourth tumors were discovered in the abdomen. Lying above and behind the second portion of the duodenum, and adherent to the inferior vena cava above the origin of the renal veins, was an oval mass measuring 7 by 6 by 4 cm. This was covered by a smooth red-tan membrane containing numerous dilated veins. The cut surface exhibited extremely tough tissue divided into two distinct areas: a pink-tan cortical region, 1 cm. in thickness, completely surrounding a central area composed of dense white fibrous tissue containing flecks of calcareous material. The other abdominal lesion was a pink-tan nodule, 1 cm. in diameter, which lay adjacent to but entirely separate from the larger tumor.

In the lungs there was evidence of acute, bilateral, confluent bronchopneumonia with abscesses containing aspirated food fragments. The liver was congested, and the spleen, which weighed 390 gm., showed diffuse fibrosis.

Microscopic Description

Microscopic examination of tissue blocks fixed in Helly's fluid and stained with hematoxylin and eosin, revealed similar histologic patterns in the 4 individual tumors. The characteristic pattern consisted of nests of polygonal cells with moderate amounts of deep pink cytoplasm and small oval hyperchromatic nuclei. The cell nests were surrounded by strands of connective tissue of variable width. In the cervical mass, some of the cell clusters were encompassed by wide bands of fibrous tissue, whereas other tumor cells were arranged in irregular clumps separated by narrow bands of connective tissue (Fig. 3). The latter pattern predominated in the intracranial and small abdominal tumors (Figs. 2 and 7). In sections from the dense central area of the larger abdominal mass, wide sheets of dense fibrous tissue were seen; in this a few clusters of neoplastic cells were embedded (Fig. 5).

Occasional clumps of yellow-brown pigment were scattered in the fibrous tissue in all the tumors. These gave a positive reaction for

iron. Moderate-sized nerve fibers were present in the capsules of the cervical lesion and the larger abdominal mass. Sections from all the tumors stained for reticulum fibers showed strands of delicate fibrils surrounding the clusters of neoplastic cells (Figs. 4, 6 and 8).

Histochemical Observations

Several histochemical procedures for the detection of enzymes, carbohydrates, and lipids were performed on sections of the tumor tissue.

Fresh frozen sections stained for esterase activity by the method of Ravin, Zacks and Seligman[11] revealed moderate cytoplasmic total esterase activity (Fig. 9). After physostigmine inhibition, marked suppression of staining occurred, indicating the presence of significant cholinesterase activity. No attempt was made to distinguish serum cholinesterase from acetylcholinesterase. Fresh frozen and formalin-fixed sections stained for alkaline phosphatase by the Gomori method[12] disclosed no enzymatic activity in the tumor cells. Endothelial cells in the stroma, however, were stained.

Formalin-fixed sections embedded in paraffin and stained by the periodic acid-Schiff method of McManus[13] exhibited rare cells with intracytoplasmic PAS-positive, diastase-resistant granules. These were believed to be macrophages. The cytoplasm of the neoplastic cells was not stained. Formalin-fixed, frozen sections stained for neutral fat with Sudan III showed occasional small intracytoplasmic fat droplets in the tumor cells and larger droplets in scattered stromal macrophages.

By means of the Baker[14] procedure for determining phospholipids, the cytoplasm of the tumor cells was seen to contain minute, deep blue-gray granules (Fig. 10). Control sections cut from blocks which had previously been extracted with hot pyridine revealed a few small gray-black cytoplasmic granules in many tumor cells. No metachromasia appeared in frozen sections stained with toluidine blue. In sections prepared by the chromate and iodate methods of Hillarp and Hökfelt[15] no chromaffin granules were observed. The adrenochrome method of Sevki[16] was negative.

Blocks of frozen tissue from both the cervical and the abdominal neoplasms were extracted and tested for the presence of epinephrine and norepinephrine* by the fluorometric method of Sobel and Henry.[17] Neither mass contained epinephrine. Both contained very small amounts of norepinephrine (0.5 μg. per gm.).

* Grateful acknowledgment is due to Dr. Charles DuToit in whose laboratory the epinephrine and norepinephrine assays on the tumors were performed and to Dr. Philip M. LeCompte who reviewed the slides.

Discussion

As stated in the introduction, neoplasms resembling the carotid body tumor have been found in sites unrelated to the carotid and aortic bodies. Instances of lesions resembling carotid body tumors microscopically have now been reported arising in the glomus jugulare, orbit, pancreas, and organs of Zuckerkandl. The present case illustrates multiple tumors including two which were retroperitoneal.

The occurrence of multiple similar neoplasms[3,5,7,8] raises the possibility of metastases from malignant carotid body tumors. The lesions found in the present case, however, showed no evidence of malignancy or invasion of surrounding structures upon microscopic examination. Only a few acceptable examples of metastases to mediastinal lymph nodes, lung and bone are recorded in this type of neoplasm.[18] A diagnosis of malignancy determined on the basis of the microscopic characteristics of a primary tumor, in the absence of demonstrable metastasis, must be considered inconclusive.[19] It is believed, therefore, that chemodectomas arise independently in multiple foci.[5,7,8,20]

The derivation of carotid body tissue has been the subject of considerable discussion for many years. The acceptance earlier by Kohn[23,24] of the sympathetic origin of this tissue has been questioned by Hollinshead.[25] The current evidence provided by Heymans and Bouckaert,[26] Schmidt and Comroe[27] and others, suggests that the carotid body is a chemoceptor and not a gland of internal secretion. The hypothesis of origin from sympathetic nerve tissue is based primarily on incompletely established embryologic evidence and also on the occasional reports of positive chromaffin reaction in carotid body tumors.

Attempts to demonstrate a chromaffin reaction by several methods in the present case all failed. However, even if found, the chromaffin reaction is inadequate evidence to indicate the presence of epinephrine. Modern views of the chromaffin reaction[15,28] indicate that epinephrine is oxidized to adrenochrome and then polymerized to form brown pigment in the presence of oxidants such as dichromate or periodate. This reaction occurs with polyphenols, aminophenols and ortho- and para-polyamines and therefore is not specific for epinephrine.

Epinephrine could not be demonstrated by a chemical method in the case reported by Cragg,[6] and two bioassays of carotid body tumors by LeCompte[20] revealed negligible vasopressor activity in one case and moderate activity in the second. The chemical nature of the substance responsible for vasopressor activity was not determined. In the present case, a sensitive fluorometric method[17] failed to dis-

close epinephrine in either the neck or the abdominal lesions. A small amount of norepinephrine, probably associated with nerve endings, was found. The lack of specific chemical identification of epinephrine in any of the recorded carotid body tumors indicates that chemodectomas lack epinephrine and are not clearly of sympathetic origin. For these reasons, the term "chemodectoma"[9] seems preferable to "nonchromaffin paraganglioma" or "carotid body tumor" for tumors arising in sites other than the carotid or aortic bodies, even though these other sites have not been proved to contain functioning chemoceptor tissue. The fortuitous occurrence of abdominal chemodectomas may indicate the presence of chemoceptor tissue in sites not previously recognized as part of the chemoceptor system.

The histochemical detection of cholinesterase activity in the cells of a chemodectoma is of interest although it fails to shed light on the origin of these cells. By chemical means, Hollinshead and Sawyer[29] demonstrated small amounts of acetylcholinesterase and serum cholinesterase in the carotid body cells of the cat. The serum type cholinesterase was demonstrated histochemically in the carotid body of the cat by Koelle.[30] These observations fail to exclude or confirm a sympathetic origin of this tissue, because adrenal medulla and sympathetic ganglia also contain cholinesterase.[29,31]

Another previously undescribed observation made in the present histochemical survey, was the finding of variable numbers of phospholipid granules in the cytoplasm of the tumor cells. Granular cytoplasm in carotid body tumor cells has been noted frequently.[20] LeCompte attributed the existence of variable granularity in part to technical manipulation. However, de Castro[32] believed that apparent chromaffin reactions in the carotid body were due to granular lipoid substances in the cells. Hollinshead[25] confirmed the presence of cytoplasmic granules; however, he believed they were not lipids but might be mitochondria. More recent studies have indicated that mitochondria are rich in phospholipid,[33] and therefore the phospholipid granules demonstrated by the Baker method may well be mitochondria.

Summary

A 41-year-old man was found to have 4 chemodectomas, one in the right carotid body, one in the left glomus jugulare, and two in the retroperitoneal region. The probable origin of chemodectomas in other sites than the neck suggests the possibility of normally occurring chemoceptor tissue not previously recognized in these areas.

The origin of these tumors from other than sympathetic tissue is suggested by the absence of epinephrine.

Histochemical tests revealed the presence of cholinesterase, phospholipid and small amounts of neutral fat but no glycogen in the cytoplasm of the neoplastic cells.

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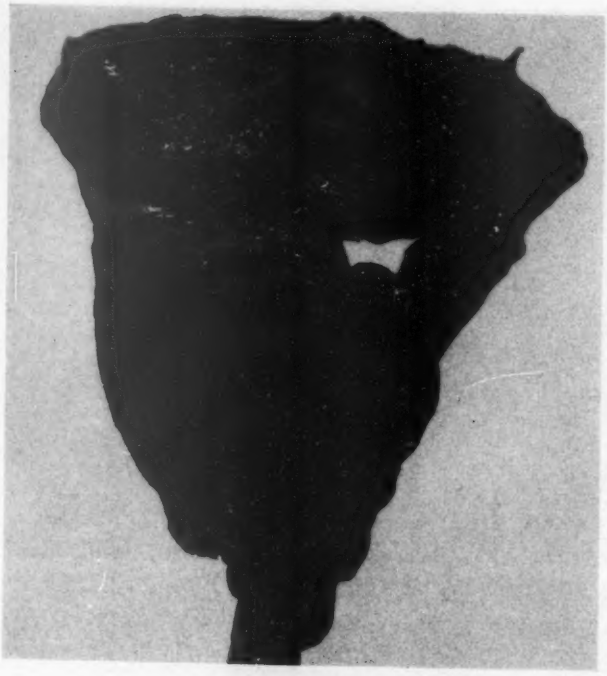
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Gross appearance of carotid body tumor.

FIG. 2. Microscopic appearance of tumor projecting from temporal bone. Hematoxylin and eosin stain. × 150.



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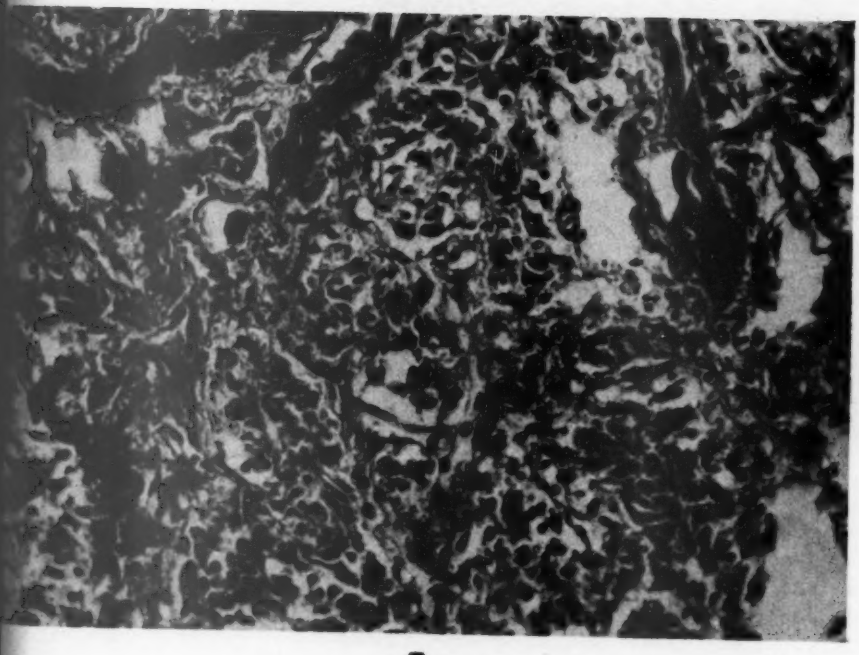

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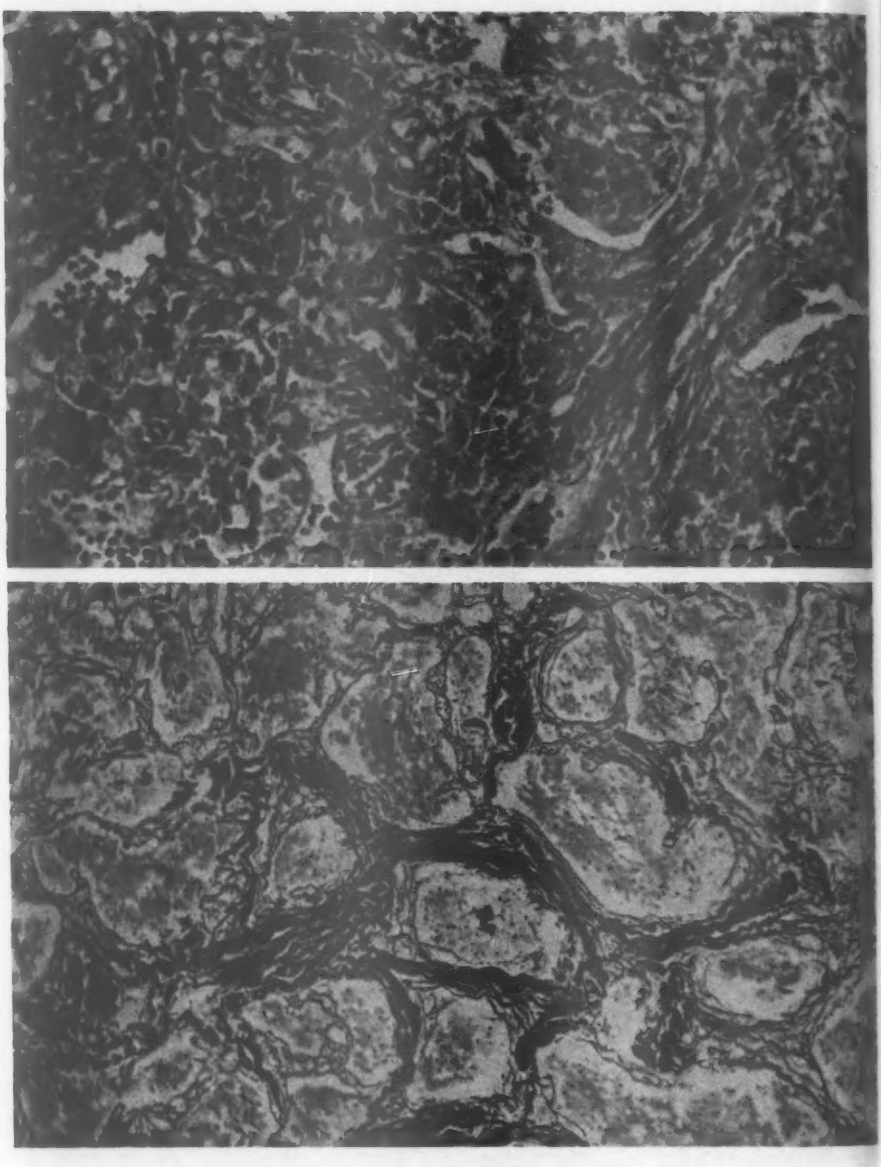
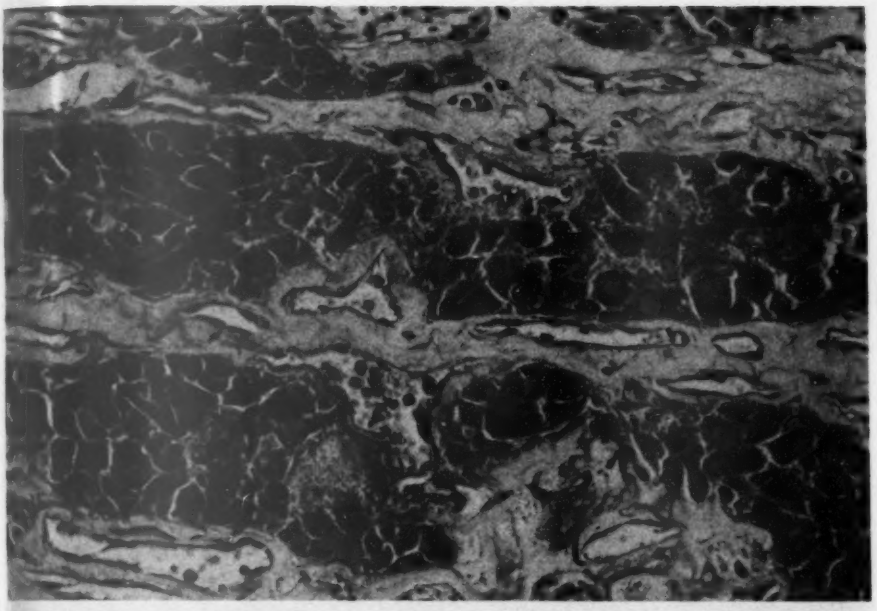


FIG. 3. Microscopic appearance of carotid body tumor. Hematoxylin and eosin stain. × 200.

FIG. 4. Carotid body tumor, showing cell clusters surrounded by dark-staining fibrils. Reticulum stain. × 200.



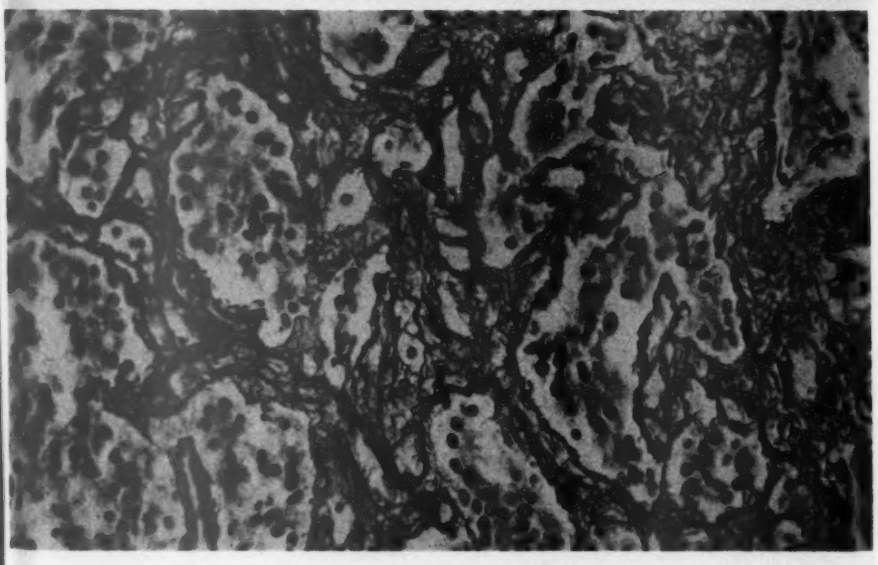
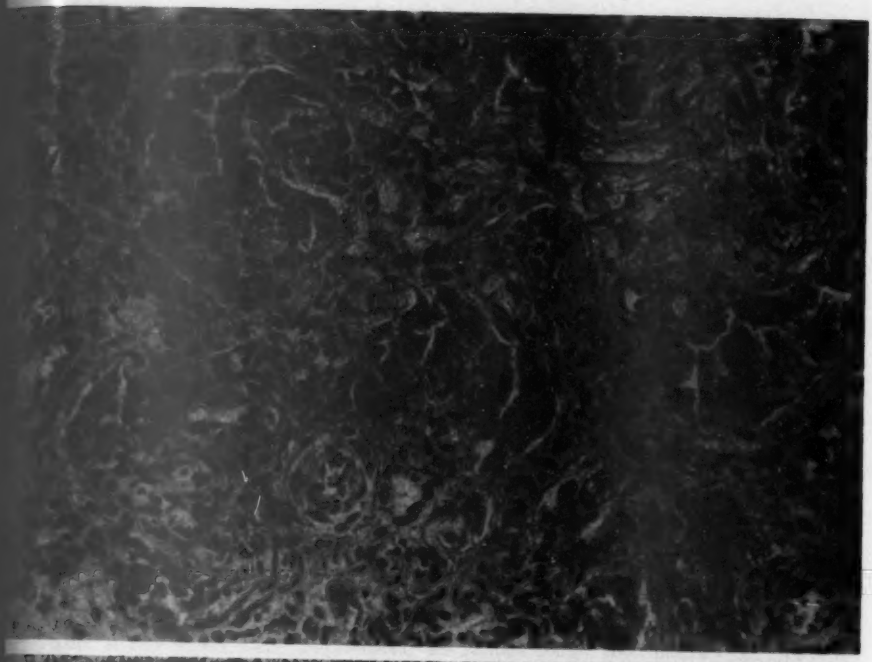


FIG. 5. Microscopic appearance of larger abdominal lesion showing cell clusters and surrounding fibrous tissue. Hematoxylin and eosin stain. × 300.

FIG. 6. Large abdominal tumor, showing cell clusters. Reticulum stain. × 200.

FIG. 7. Microscopic appearance of smaller abdominal mass. Hematoxylin and eosin stain. × 300.

FIG. 8. Smaller abdominal tumor, showing cell clusters. Reticulum stain. × 200.



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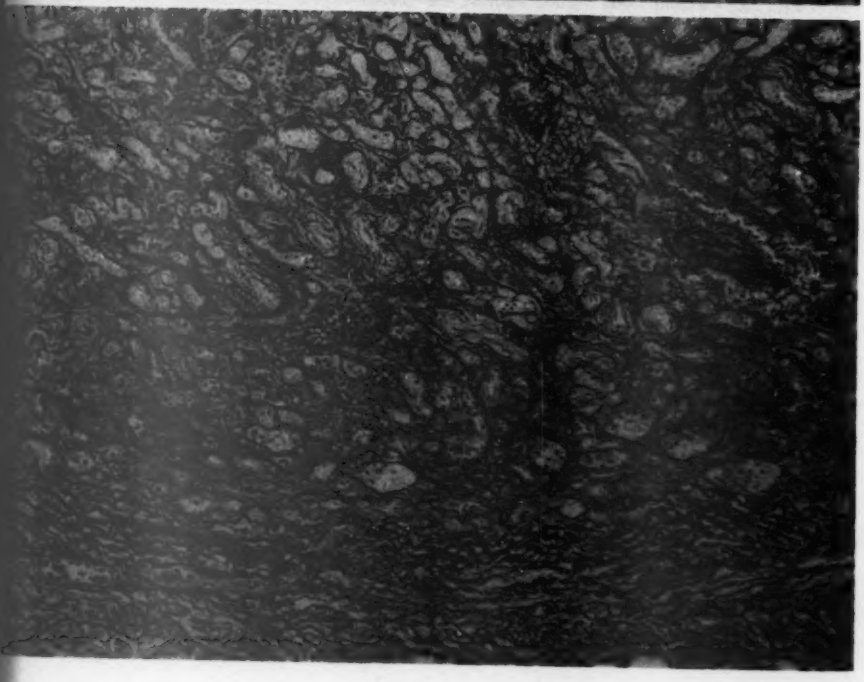

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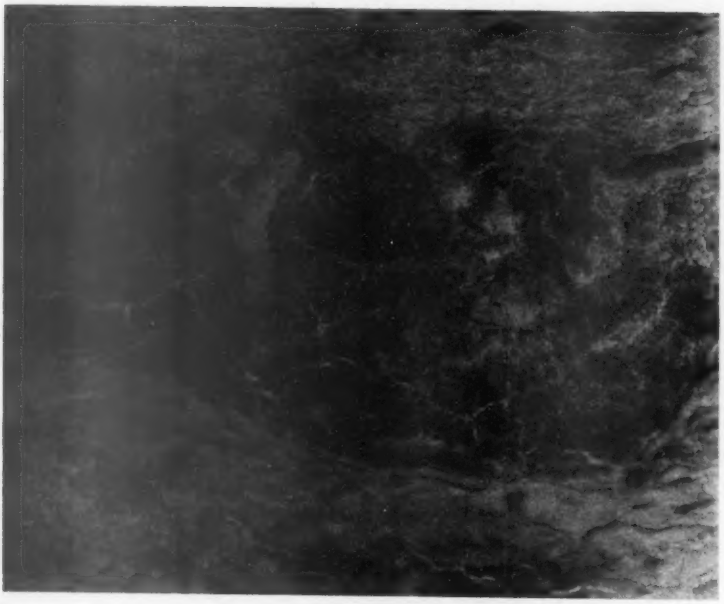
FIG. 9. Carotid body tumor, showing histochemical reaction for cholinesterase. The dark punctate granules are within the neoplastic cells, whereas the surrounding fibrous tissue is free of dye granules. × 150.

FIG. 10. Carotid body tumor stained for phospholipid. Each tumor cell is filled with dark-stained granules. The clear area in each cell represents the unstained nucleus. × 200.



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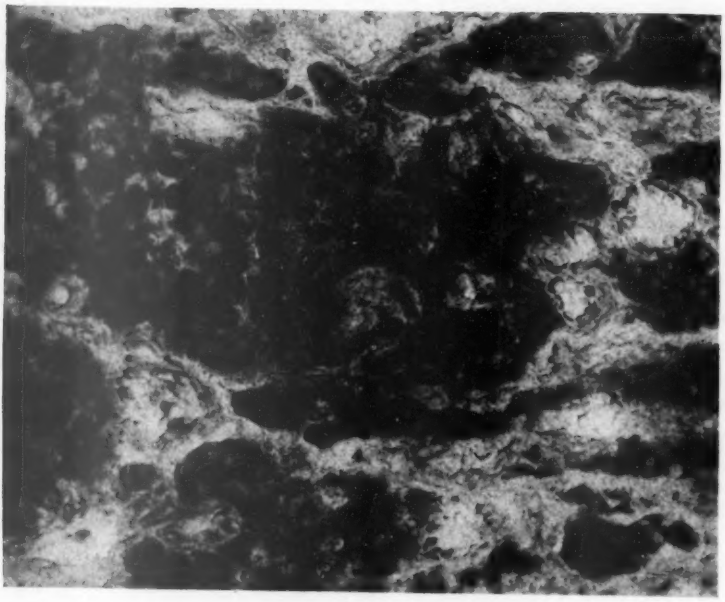

10

SPONTANEOUS TUMORS IN THE ROCHESTER STRAIN OF THE WISTAR RAT*

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The increasing demand for long term toxicity studies of rats has stimulated the interest of the pathologist and toxicologist in the spontaneous disease processes and neoplasms that arise in old rats. To evaluate the possible carcinogenic effect of compounds under study, the spontaneous tumor incidence must be known for the strain of rat being used. It is the purpose of this paper to describe 200 spontaneous tumors found in 786 albino rats† 18 to 24 months old (Table I).

From what has been written of spontaneous tumors in rats, it is evident that considerable variation exists in type and frequency of tumors in different strains and species. In one of the earlier papers on the subject, written in 1909, McCoy[1] described 103 tumors in a group of 100,000 wild rats of the species *Mus norvegicus*. Bullock and Curtis,[2] in the course of the experimental production of sarcoma of the liver in rats by feeding cat tapeworm larvae, examined 31,868 rats and found 521 primary spontaneous tumors in 489 rats. Ratcliffe,[3] in 1940, described 302 spontaneous tumors found in 273 rats of the Wistar Experimental and Stock Colonies. Saxton, Sperling, Barnes and McCay[11] reported 234 spontaneous tumors in 498 albino rats of the Osborne-Mendel (Yale) strain. Other reports have described a small series of tumors limited to one organ system or an unusually high incidence of one particular type of tumor.

MATERIAL AND METHODS

The rats used represented 11 different 2-year toxicity studies conducted over a span of 6 years. Of the 786 rats examined for tumors, 139 were fed control diets while the remainder received an experimental diet judged not to be carcinogenic as determined by comparison with simultaneous control groups. A description follows of the usual organization of a 2-year feeding study.

Groups of 30 to 50 male and 30 to 50 female rats of the Rochester strain were assembled by random sampling at the time of weaning.

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† The Rochester albino rat was obtained from the Wistar Institute in 1923.

TABLE I
Summary of Spontaneous Tumors

Tumors	Control male	Control female	18 to 24-month male	18 to 24-month female	Total
Breast					
Fibroadenoma		11		42	53
Adenoma				5	5
Cystadenoma		1		3	4
Fibroma		1		1	2
Carcinoma		1			1
Skin and subcutaneous tissue			2	2	4
Mesenteric lymph nodes	4	1	14	4	23
Lung					
Lymphoma, malignant	1		10	7	18
Fibrosarcoma				2	2
Kidney					
Papillary cystadenoma			1		1
Lipomatous hamartoma				1	1
Adrenal					
Medullary adenoma			5	5	10
Uterus					
Leiomyoma				2	2
Mesodermal tumor		1		6	7
Adenocarcinoma		1		2	3
Liver					
Undifferentiated spindle cell tumor				1	1
Ovary					
Fibroma		1		2	3
Adenocarcinoma				1	1
Testis					
Interstitial adenoma					
Unilateral	4		26		30
Bilateral	1		4		5
Pituitary adenoma	1	2	1	7	11
Alimentary tract					
Mucoid carcinoma			1		1
Adenocarcinoma		1		1	2
Squamous cell carcinoma			1		1
Thyroid adenoma			1	1	2
Lipoma			1	1	2
Miscellaneous			1	4	5
Number of rats	55	84	279	368	786
Tumor bearers	11	20	64	94	189
Number of tumors	11	21	68	100	200

Groups were matched initially for body weight and maintained thereafter on the control and experimental diets for 2 years. In most instances there were 4 equal groups of each sex, one control group and 3 dietary levels of the compound being tested. The control diet consisted of Purina Fox Chow meal. For the experimental diet the test product was mixed with control food by machine. Rats were generally housed 5 to a cage in metal cages with wire doors; the bottom pan was filled with wood shavings. Food and drinking water (Rochester tap water) were supplied *ad libitum*. A program of serial sacrifice was instituted whereby a small number (10 per cent) were sacrificed and necropsied at 6 month intervals. Microscopic examination was carried out upon tissue from all major organ systems. The animals were weighed weekly throughout the experiment; at necropsy, individual organ weights were recorded. Animals which died naturally during the first 18 months of the study were necropsied to determine the cause of death, but sections were seldom taken. During the last 6 months (18 to 24 months), however, whenever an unusually high mortality had been noted, moribund animals were sacrificed and complete gross and microscopic examinations were performed on each to insure that a sufficient number of tissues would be available at the termination of the study for meaningful comparison. The animals were sacrificed by decapitation and tissue samples were taken of heart, lung, spleen, stomach, small intestine, large intestine, pancreas, liver, adrenal, both kidneys, bladder, gonad, bone marrow, brain and thyroid. In addition, any tumor or unusual appearing tissue was sectioned for histologic study. Tissues were fixed in Zenker's solution with acetic acid, and sections were stained with hematoxylin and eosin.

Results

Tumors of Mammary Gland

Spontaneous mammary tumors constituted the largest single group of neoplasms in this series, accounting for 65 of the 200 tumors. It was difficult to draw comparisons with previous studies recorded in the literature because of variation in the criteria for selection. However, it was evident that mammary tumors were prevalent in many strains. Bullock and Curtis[2] reported an incidence of mammary tumors of 18 per cent among 521 tumors. Ratcliffe[3] reported an incidence of 75 per cent among 302 tumors found in rats at the Wistar Institute. The selection of rats in the latter study depended upon visual external recognition of body tumors; consequently mam-

mary tumors would be expected to constitute a greater proportion. Bryan, Klinck, and Wolfe[4] reported an unusually high incidence of 51 per cent among 51 female rats of their colony. This study was followed by further work on the same colony by Wright, Klinck, and Wolfe[5] showing a reduced incidence of breast neoplasms, with approximately 11 per cent among 1,827 rats over 5 months of age.

By the nature of the normal concentration of mammary glands at the cephalad and caudad ends of the mammary line, mammary neoplasms were most commonly encountered in these regions. Wright and co-workers[5] noted that roughly two thirds of the mammary tumors they encountered were in the axillary regions of the foreleg while the remainder were near the groin. There was about equal distribution between the right and left sides.

In the study reported here, no record of tumor site was kept. Most of the neoplasms were single; a small number of animals had two separate neoplasms and one rat had 3 fibroadenomas. The lesions varied greatly in size, some measuring less than a centimeter while the largest tumor weighed 256 gm. It appeared that these tumors were well tolerated by the animal until they approached half the total weight of the rat; thereafter the rat began to lose weight and became moribund.

Mammary tumors usually appeared as ovoid, spherical or occasionally discoid masses; they were covered by the skin but usually not attached to it. The tumors were nodular, firm, and elastic to compression. Some of the larger lesions showed loss of hair at points of friction, or ulceration with secondary infection. The bearers of large tumors frequently showed marked emaciation and body deformity and had locomotor difficulties. When exposed, the lesions were lobulated and surrounded by a capsule. The cut surfaces pouted above the capsule and gave the classical whorled silk appearance observed in leiomyomas of the uterus. Some of the larger tumors exhibited focal hemorrhage, necrosis, and cystic degeneration probably resulting from insufficient blood supply.

Histologically, the tumors ranged from fibromas consisting entirely of connective tissue to adenomas of solid glandular content (Fig. 2). The fibroma of mammary origin differed from dermal fibromas in the presence of rare isolated ducts and glands in the stroma. The intermediate range was represented by various combinations of glandular and stromal elements designated as fibroadenomas (Fig. 2). Depending on the arrangement of the glandular elements, the lesions were designated as cystic or papillary. Of the 65 breast tumors, 4 were classified as cystadenomas (Fig. 3), and one of these showed a

papillary arrangement of the glandular elements (Fig. 4). In some cases cystic, papillary, fibrous and adenomatous variations were all seen in different portions of the same tumor. Wright and co-workers[5] described intracanalicular and pericanalicular fibroadenomas; these were not seen in our animals.

The criterion for malignant neoplasm was the demonstration of metastasis. The sole example of malignant tumor in this group was a lesion histologically indistinguishable from the benign cystadenoma, but a metastasis was found in the lung.

Tumors of Lymph Nodes

A group of lymph nodes draining the ascending colon and situated in the ileo-colic mesentery was the site of one of the most common tumors encountered in this series, a malignant lymphoma (Figs. 5 and 6). The tumor has been variously known as polymorphous cell sarcoma, lymphoblastic lymphosarcoma, and reticulum cell sarcoma, all of which fall under the same family heading of malignant lymphoma. Although lymphomas were most commonly seen in cecal lymph nodes and occasionally as metastatic lesions disseminated over peritoneal surfaces, the peribronchial lymphoid tissue also was a frequent site for this tumor. Some lymph nodes contained a small nidus of malignant neoplasm in one portion of the node. In other instances there were varying degrees of alteration of architecture ranging to complete replacement of the node by neoplasm. Total replacement occurred in most cases. Cecal lymph nodes and peribronchial lymphoid tissue have been found to be frequent sites of lymphoid hyperplasia, accompanying ulcerative cecitis[6] in the former and bronchiectasis, bronchitis and bronchopneumonia in the latter.[7] The high correlation between the incidence of lymphoid hyperplasia and occurrence of tumor has led to speculation concerning the character of the neoplasm and the role played by inflammation in its production.

Saxton and co-workers[11] reported 91 cases of lymphosarcoma of the lung among 234 tumors. Bullock and Curtis[2] found that malignant lymphoma accounted for 78 of the 521 tumors in their group of animals. Of the 200 tumors examined in this study, 41 were of lymphomatous nature. In 23 rats cecal nodes were the seat of the lesion; some of these rats had lymphoma in the pulmonary lymphoid tissue as well. Lymphoma of peribronchial origin alone was seen in 18 rats. The cecal lymphomas sometimes occurred as nodular masses in the mesentery, attaining a size of several centimeters in diameter. They were contiguous with the cecum or appeared as several separate

nodules. In some cases, the tumor invaded the wall of the cecum, or by peritoneal metastasis, reached the serous surfaces of stomach, pancreas, adrenals, kidneys, liver and spleen. In one case, lymphoma was found to be widely distributed throughout the liver with compression and necrosis of hepatic tissue. Lymphomatous lesions in the lung were not easily recognized grossly and were frequently mistaken for inflammatory lesions.

To gross examination, the lymphoma appeared gray-white and soft, and the masses were prone to develop necrosis, cystic degeneration and hemorrhage. The histologic appearance was similar in all and was characterized by large loosely arranged cells containing scant cytoplasm and large, irregular nuclei of varied size. The chromatin was heavy and irregularly clumped. Bizarre mitotic figures were numerous and nucleoli were large and frequently multiple. The nuclear membrane was heavy and the nuclei were round, oval, lobulated, or polyhedral in configuration. The cells formed no consistent pattern but were separated by a vascularized loose connective tissue (Figs. 5 and 6).

Tumors of the Testis

Reports of testicular neoplasm in rats are uncommon. Bullock and Curtis[2] reported one testicular tumor which proved to be similar to those encountered in our colony. Gilman, Gilbert and Spence[8] referred to a high incidence of interstitial cell adenomas of the testis in rats of the Wistar strain. Saxton and co-workers[11] reported one interstitial cell tumor and one embryonal carcinoma of the testis.

Testicular adenomas comprised a substantial portion of the present series. There were 35 of these among the 200 tumors. The lesions were generally small and did not extend beyond the confines of the *tunica albuginea*. When the existence of neoplasm was recognized grossly, the involved testis was brown and soft. The contralateral testis was occasionally atrophic, but in 5 of the 35 cases the opposite testis also contained an adenoma.

The tumors appeared to originate from small nests of hyperplastic interstitial cells which presumably multiplied gradually, causing compression and atrophy of adjacent tubules. The adenomas were formed of solid sheets of cells with distinct outlines and abundant acidophilic cytoplasm filled with numerous small vacuoles (Figs. 7 and 8). In some areas, the vacuoles were prominent, giving the cell the foamy appearance of a lipid-filled macrophage (Figs. 9 and 10). In other regions, the vacuoles were very small and indistinct. Nuclei were uniform in size and had dark nuclear membranes. Chromatin was not prominent; occasional mitotic figures were noted. Scattered de-

posits of yellowish-brown pigment were evident in many of the smaller tumors.

In the larger tumors, two different cell types were evident. The characteristic cell with foamy cytoplasm formed nodules of varied sizes, surrounded by smaller compact cells with scanty, ill-defined cytoplasm and uniform small dark nuclei. Collections of golden-brown pigment were numerous in these peripheral areas. Large vascular sinusoids appeared within the tumors and red cell extravasation was a common finding. No extension beyond the testis was encountered (Figs. 9 and 10).

Tumors of the Adrenal Glands

Bullock and Curtis[2] described only 4 adrenal tumors resembling those encountered in this study. Staemmler[9] observed focal hyperplasia of the adrenal medulla in rats but considered this to be a result of nicotine poisoning. Yeakel[10] examined the adrenal glands of 16 female and 15 male rats of the Wistar strain and 3 male and 3 female gray Norway rats, all over 700 days old. Thirty-six glands from females of both species were available for study. Hyperplastic changes of the medulla were noted in 11 per cent. Fifty-one per cent of the 35 glands from males of both species showed similar hyperplasia. Saxton, Sperling, Barnes and McCoy[11] reported the occurrence of 5 tumors of the adrenal glands in 498 rats. Recently, Gilman and coworkers[8] reported a study of 167 male and 189 female rats from the Wistar Institute with an age range from one month to more than 2½ years. The adrenal glands of these rats were carefully examined microscopically. The incidence of medullary hyperplasia or adenoma varied between 50 and 76 per cent in female rats 13 to 30 months old, and 82 to 86 per cent in male rats in the same age range. These investigators used the term "phaeochromocytoma" to designate the tumors of the medulla and suggested a possible relationship to the high incidence of chronic myocarditis, hepatic necrosis, and endocrine tumors (interstitial adenoma of testis, fibroadenoma of breast, thyroid adenoma, and pituitary adenoma) found in their series.

Ten medullary adenomas were encountered among our own animals. In most cases, the tumor was not recognized on gross examination. The frequency with which the adrenals of old rats were enlarged, cystic, and sometimes hemorrhagic as the result of acute infections made size a dubious criterion in detecting small adenomas.

The smallest adenomas appeared as small clusters of intensely basophilic cells in the medulla. The sparse cytoplasm and marked basophilic staining of the nuclei were in distinct contrast to adjacent

medullary elements (Fig. 11). The larger tumors formed nodular clusters about sinusoids; occasionally there were multiple clusters in separate portions of the medulla. The largest medullary tumors caused compression and narrowing of the cortex. Indeed, the largest in this series, measuring 1 cm. in diameter, had penetrated the cortex and capsule into the adjacent perirenal fat. In this, the nuclei were larger, vesicular and less basophilic, and the cytoplasm more abundant and more clearly demarcated. The difference in cell type was not a regular feature and considerable variation was encountered. The cells of the small adenomas were arranged in solid cords separated by a delicate capillary network, whereas in the larger tumors the vascular network consisted of large sinusoids and venous lakes. Frank invasion was not observed in this study, although Gilman and co-workers[8] reported direct extension of the tumor into the inferior vena cava and embolization to the lung.

The necropsy sections and the organ:body weight ratios of the rats in this series were analyzed in an effort to establish a correlation between possible pressor substance secretion by the adrenal tumors and cardiovascular alterations. All but two of the heart weight:body weight ratios were within or below the normal range. In the two rats in which this range was exceeded, only microscopic adenomas were present, and there were no other necropsy findings to indicate either an endocrinopathy or cardiovascular disease.

Another tumor of the adrenal, seen in a rat under 6 months of age, has been included in this report because of its unusual nature. This lesion most nearly resembled a neuroblastoma. It originated in the left adrenal medulla and invaded the spleen and adjacent peritoneum. The splenic metastases consisted of several nodules of friable gray-white tissue which varied from 0.6 to 1.8 cm. in diameter. The largest nodule was hemorrhagic and necrotic and accounted for the presence of free blood in the peritoneal cavity. The tumor was cellular and its nuclei were variable in size and shape. In a few areas, tumor cells were arranged in rosettelike fashion.

Tumors of the Uterus

Bullock and Curtis[2] found the uterus of the rat to be a common site of neoplasm. They reported 48 tumors of this organ with the following structure: "leiomyoma, 4; myosarcoma, 2; fibrosarcoma, 2; polymorphous cell sarcoma, 2; spindle cell sarcoma, 1; mixed cell sarcoma, 16; papilloma, 1; squamous cell epithelioma, 10; adenocarcinoma, 4; adenoacanthoma, 1; carcinoma, 1; carcinosarcoma, 4." Ratcliffe[3] reported 10 tumors of the uterus.

In the present series, 12 of the 200 tumors were of uterine origin and were characterized as: leiomyoma, 2; mesodermal tumor, 7; adenocarcinoma of the endometrium, 3. Most of these lesions were not described grossly. The leiomyoma was a well circumscribed nodule composed of interlacing bundles of smooth muscle and fibrous tissue with considerable uniformity of cell type.

The group of mesodermal tumors showed variation in cell type and numerous cystic areas (Figs. 12 to 15). The predominant cells were primitive mesenchymal elements with oval, stellate, and spindle-shaped nuclei. Portions of some mesodermal tumors were composed of bizarre giant multinucleated cell forms. There was considerable variability of cell type and no correlation with the occurrence of metastasis.

The cases of adenocarcinoma of the endometrium showed widespread metastases to the peritoneum, lung, and liver. The tumors exhibited considerable necrosis and marked acute inflammatory reaction (Fig. 16).

Tumors of the Kidney

The series reported by Bullock and Curtis[2] included 6 embryonal carcinomas, 1 carcinoma, and 1 sarcoma of the kidney. Eker[12] reported a group of renal adenomas in Wistar rats 6 to 16 months of age. These appeared as simple cysts, papillary cystadenomas, solid eosinophilic adenomas, and solid basophilic tubular adenomas and varied in size from barely visible nodules to 0.8 cm. In Eker's series, the adenomas appeared to arise from tubules, and none contained glomeruli.

In the present series there were only 3 renal tumors. One was a papillary adenoma of the cortex which was not recognized grossly and appeared microscopically as a small cystic space lined by papillary invaginations of plump epithelial cells. A second lesion designated as a lipomatous hamartoma showed numerous reduplicated folds of pelvic epithelium. The cortex and medulla of one portion of the kidney were replaced by fat that blended with normal appearing renal parenchyma on either side (Fig. 17). Isolated tubules and glomeruli were present within the fatty portion. A third tumor was included in this report because of its infrequent occurrence even though the rat was only slightly over one year of age. This was an embryonal carcinoma of the upper pole of the left kidney. It measured 0.3 by 1.8 by 1.5 cm., was grayish-brown and did not appear to have invaded adjacent structures. Microscopically, the tumor showed structures resembling tubules and glomeruli (Fig. 18).

Tumors of the Pituitary

Chromophobe adenomas of the pituitary accounted for 92 of the 234 spontaneous tumors reported by Saxton and co-workers.[11]

Eleven adenomas of the pituitary were identified in this series. No gross description of these tumors was recorded. All the lesions were similar histologically although there was variation in vascularity (Fig. 19). The tumors were composed of cords of plump basophilic cells separated by a delicate reticulum and congested vascular spaces. The cytoplasm was scant and poorly defined. Nuclei were large, oval or round and had prominent nuclear membranes with a light sprinkling of chromatin. Many nuclei contained a prominent central nucleolus. Deposits of brown pigment resembling hemosiderin were present in the cytoplasm of some of the cells and within vascular sinusoids. There was no invasion of adjacent structures. Mann's stain showed no chromophilic element in these adenomas.

Tumors of the Thyroid Gland

The 2 thyroid adenomas encountered may not accurately reflect the incidence of thyroid neoplasm since sections of thyroid were made in only 24 per cent of the necropsies. The tumors of the thyroid gland reported by Bullock and Curtis[2] included 1 adenoma and 1 carcinoma.

One of the adenomas consisted of solid cords and tubes of uniform large cells separated by delicate strands of connective tissue. The cytoplasm was abundant, granular, and pink staining, and the nuclei were large and vesicular. Small amounts of colloid were present in portions of the adenoma (Fig. 20). A second well-circumscribed adenoma revealed marked compression of the acini in a narrow rim of remaining thyroid about the periphery of the nodule. The tumor was very similar to the first example.

Tumors of the Alimentary Tract

Gastrointestinal neoplasms were not common. Bullock and Curtis[2] reported 1 adenocarcinoma of the stomach, 10 cecal tumors, 1 fibroma of the jejunum, and 9 sarcomas of the stomach. Willis[12] reported 2 examples of adenocarcinoma of the colon in albino rats. Saxton and co-workers[11] reported one squamous cell carcinoma of the stomach.

Four neoplasms of the alimentary tract were identified in this series. A squamous cell carcinoma of the esophagus was composed of nests of large irregular squamous cells which invaded peri-esophageal tissues. Pearl formation and desmoplasia were prominent features

of this tumor (Fig. 21). A second tumor was an adenocarcinoma of the colon which almost completely filled the lumen and invaded the wall and mesenteric fat. Focal areas of necrosis were accompanied by large numbers of acute inflammatory cells. Neoplastic acini were irregular and lined by pseudostratified columnar epithelium with large, hyperchromatic nuclei and numerous mitotic figures (Fig. 22). A third tumor was an adenocarcinoma of the stomach which invaded the adjacent mesentery. There were large areas of necrosis, and neoplastic acini were composed of pleomorphic cells with hyperchromatic nuclei. Bizarre mitotic figures were numerous. A mucoid carcinoma of the stomach was the fourth neoplasm. The tumor invaded the stomach wall and was characterized by large amounts of mucin, scattered signet ring cells and occasional acini (Fig. 23).

Tumors of the Ovary

Bullock and Curtis[2] reported 5 tumors of the ovary: a fibroma, a bilateral sarcoma, and 3 cystadenocarcinomas. Ratcliffe[8] found 3 carcinomas of the ovary. Saxton and co-workers[11] reported 5 adenomas and 2 fibromas of the ovary.

In the present series there were 3 neoplasms. One was a fibroma, not recognized grossly. This tumor was composed of large cells with little or no cytoplasm and moderately uniform oval or round nuclei. Occasional mitotic figures were seen. A second fibroma, similar to the first, measured over 2 cm. in diameter and showed large areas of necrosis in its center. The third tumor was a papillary serous cystadenocarcinoma, 2 cm. in diameter. The neoplasm was composed of branching papillary septae covered by cuboidal and columnar epithelium (Fig. 24). Peritoneal implants had occurred.

Tumors of Skin and Subcutaneous Tissue

Both benign and malignant neoplasms of skin and subcutaneous tissue comprise a substantial number of spontaneous rat tumors reported by other investigators. Bullock and Curtis[2] reported 121 tumors of skin and subcutaneous tissue among 521 spontaneous neoplasms.

Of the 200 tumors in this series, only 4 originated in the integument. One, a fibroma of the chest wall, was composed of wavy collagen bundles and sparse spindle cells with long tapering cytoplasmic processes. Occasional leukocytes were scattered through the tumor. A dermatofibrosarcoma was removed from the snout of a 24-month-old rat. The tumor was dome shaped, measured 0.8 cm., and was

covered by skin except for one small area of ulceration. It was composed of interlacing bundles of spindle cells with cigar-shaped nuclei. Invasion and destruction of skeletal muscle were seen at its base. The third subcutaneous tumor was large and consisted of a thin border of viable neoplasm, the central portion of which was composed of organizing blood clot and large vascular spaces. The cell type was thought to be of mesenchymal origin. The last tumor in this group was histologically similar to the mesodermal tumors of the uterus. It contained numerous cystic areas. Cytoplasm was indistinct, nuclei were small and relatively uniform, and mitotic figures were uncommon.

Tumors of Miscellaneous Origin

A small number of tumors could not be fitted into any category. One lipoma of the spermatic cord was found to the left of the bladder and measured 1 cm. in diameter. A second lipoma was found in the wall of the large intestine between the mucosa and the muscularis. A malignant spindle-cell tumor was present both in the lung and beneath the skin of the same rat. It was impossible to determine whether these were independent or one had metastasized from the other. The cutaneous lesion contained a large area of central infarction. In another instance a highly undifferentiated malignant neoplasm of undetermined origin had invaded liver, pancreas and mesenteric fat and was present on the serosal surfaces of spleen, bladder, and gastrointestinal tract. Tumor cells were variable in size; giant nuclear forms and multinucleated forms were numerous. Chromatin was coarse and irregularly distributed. Bizarre mitotic figures were numerous. Primary carcinoma of the liver, metastatic sarcoma, or an undifferentiated carcinoma of unknown source were possibilities considered here. In one rat with a lymphoma of the mesenteric nodes, there was metastatic squamous cell carcinoma in the frontal portion of the cerebrum. The source was undetermined.

Summary

Two hundred spontaneous tumors of the Wistar Rat have been reported and described. These occurred in 189 of 786 albino rats ranging in age from 18 to 24 months; a tumor incidence of approximately 25 per cent (Table I). Primary tumors were identified in all organs except those of the cardiovascular and locomotor systems. Approximately one third were considered malignant. The most common tumors in the order of incidence were fibroadenoma of breast, malignant lymphoma of the lung and mesenteric lymph nodes, and

interstitial cell adenoma of testis. It was of interest that many of the tumors of the rat resembled those arising in similar sites in the human host.

Comparison with previous reports of spontaneous tumors in rats showed two striking variations. In the present series there was a paucity of tumors of skin and subcutaneous tissue and an absence of thymic tumors.

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The assistance of Professor J. Lowell Orbison is acknowledged.

[*Illustrations follow*]

LEGENDS FOR FIGURES

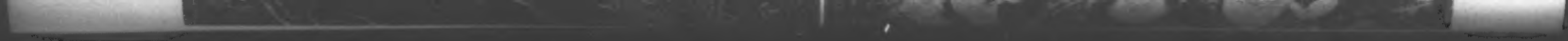
All sections shown in these photographs were stained with hematoxylin and eosin stain.

FIG. 1. Fibroadenoma of mammary gland, the most common type of spontaneous tumor in the Rochester Wistar rat. × 60.

FIG. 2. Adenoma of mammary gland. × 60.

FIG. 3. Cystadenoma of mammary gland. × 100.

FIG. 4. Papillary cystadenoma of mammary gland. × 100.



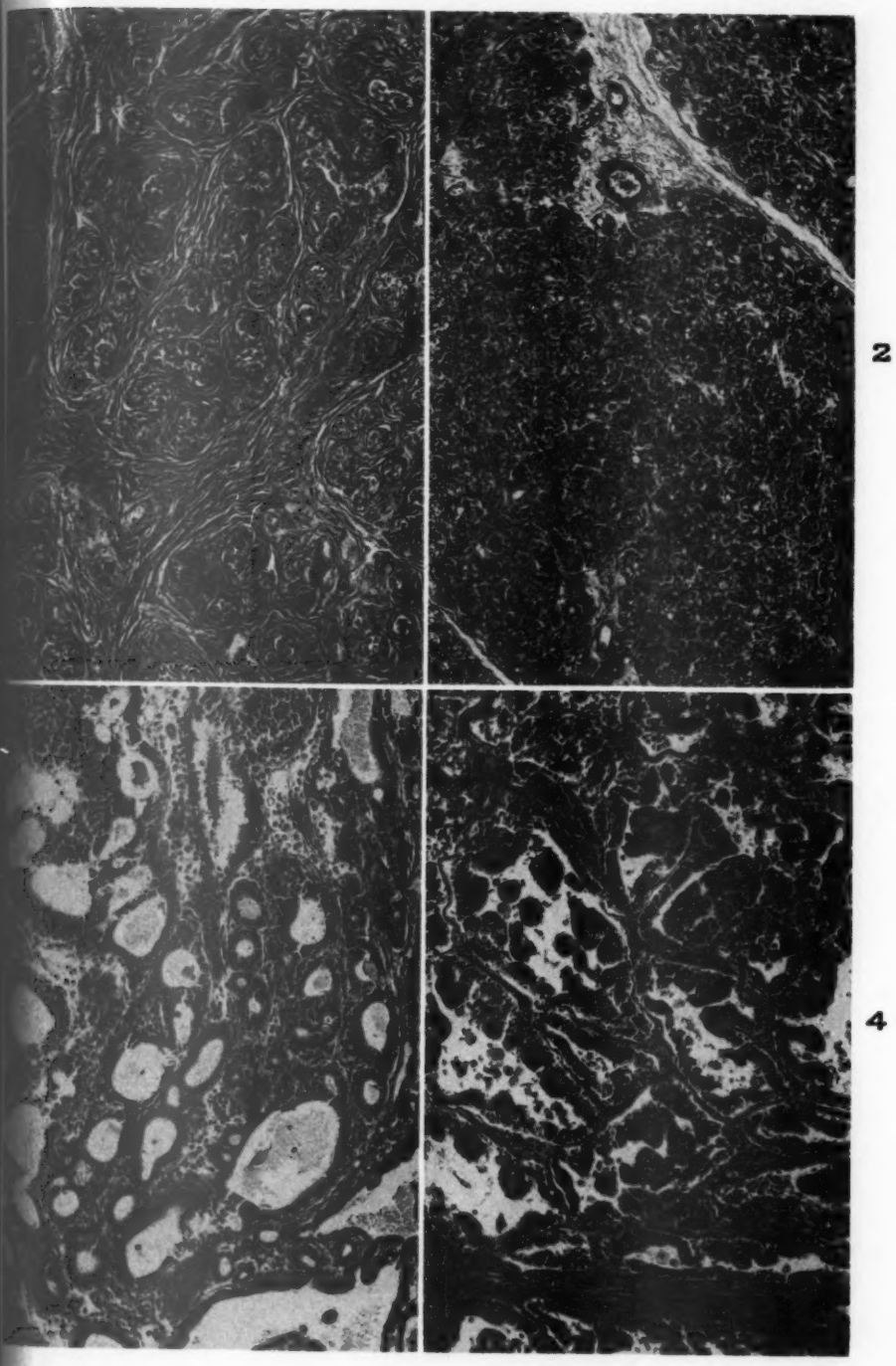


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FIG. 5. Malignant lymphoma in wall of cecum. × 60.

FIG. 6. Higher magnification of Figure 5. × 430.

FIG. 7. Small interstitial cell adenoma of testis, composed of uniform cells with acidophilic cytoplasm. × 100.

FIG. 8. Interstitial cell adenoma of testis. Higher magnification of Figure 7 × 430.

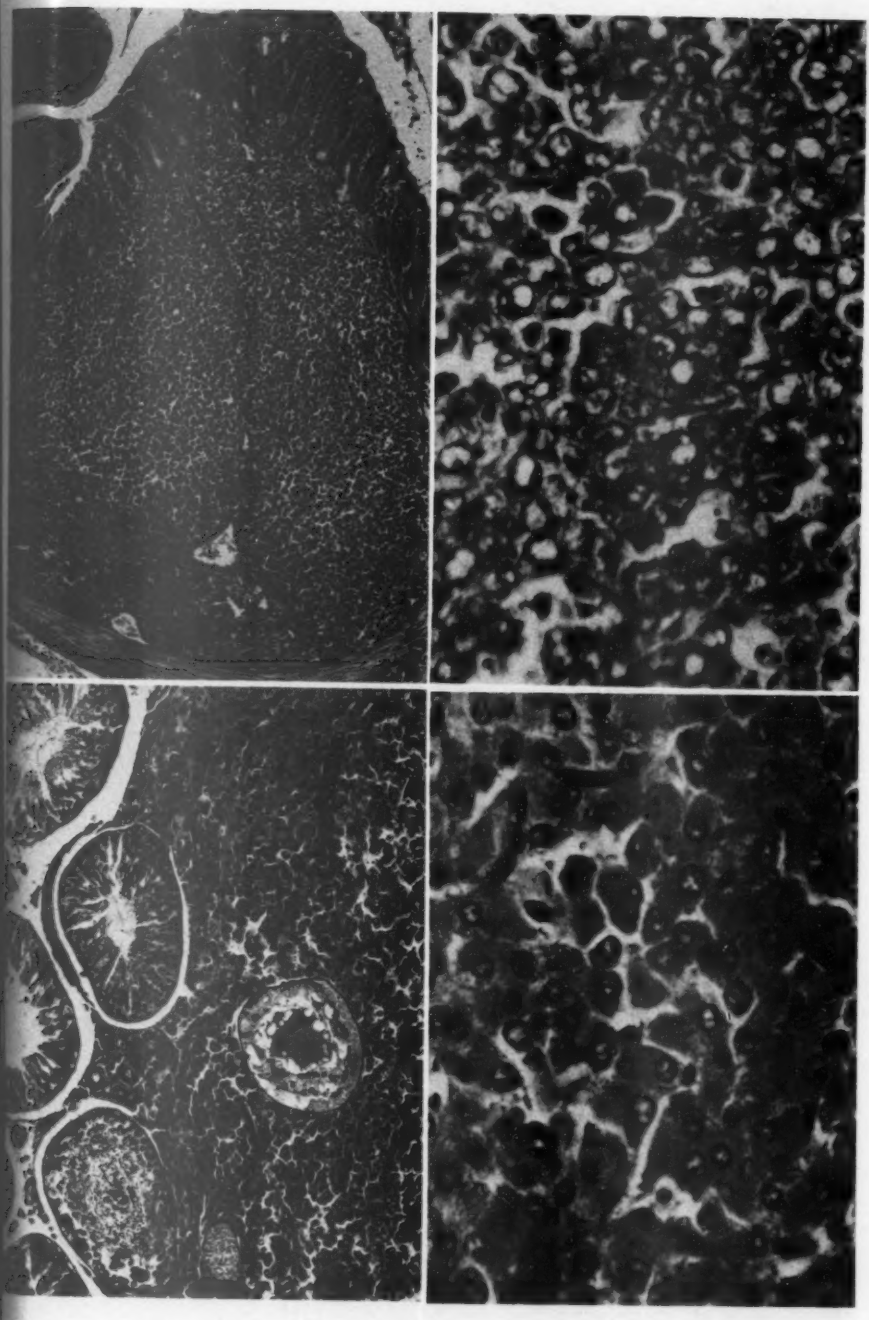

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FIG. 9. Large interstitial cell adenoma of testis illustrating two characteristic cell types. Large nodules composed of pale cells with abundant foamy cytoplasm were separated and surrounded by small, closely packed cells with deeply basophilic nuclei. × 60.

FIG. 10. Higher magnification of Figure 9. × 430.

FIG. 11. Adenoma of adrenal medulla characterized by cords and nests of cells. × 60.

FIG. 12. Mesodermal tumor of uterus showing cellular area. × 100.

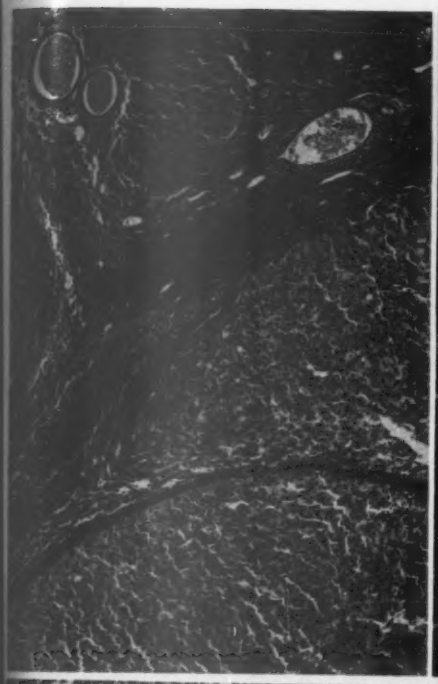

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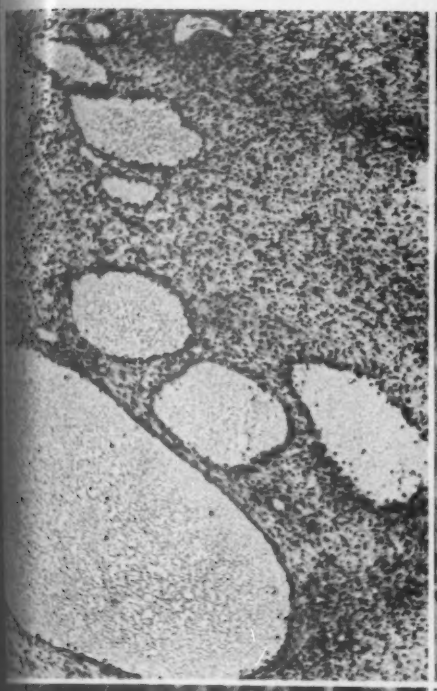
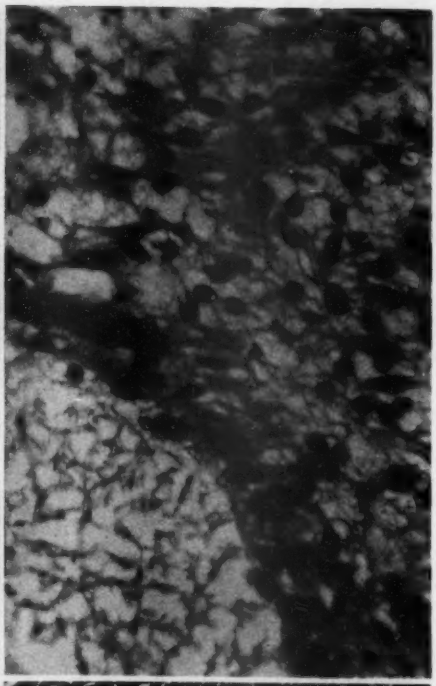
FIG. 13. Mesodermal tumor of uterus showing cystic areas. × 60.

FIG. 14. Higher magnification of Figure 13. × 430.

FIG. 15. Mesodermal tumor of uterus showing cellular pleomorphism and occasional giant nuclear forms. × 430.

FIG. 16. Adenocarcinoma of the endometrium with marked acute inflammatory cell reaction. × 100.





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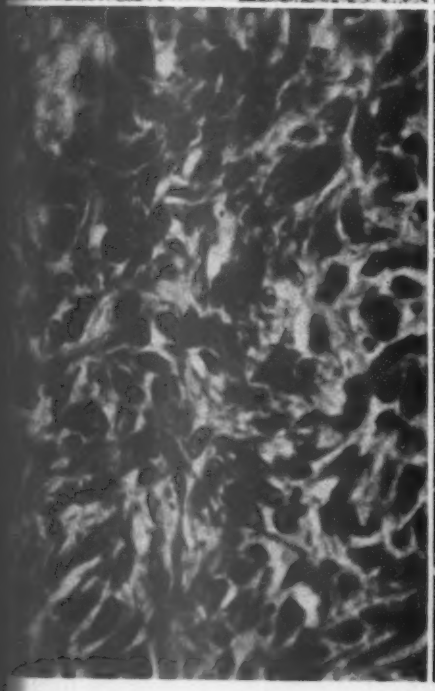

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FIG. 17. Lipomatous hamartoma of kidney. × 60.

FIG. 18. Embryonal carcinoma of kidney. × 60.

FIG. 19. Pituitary adenoma. × 430.

FIG. 20. Thyroid adenoma. × 60.

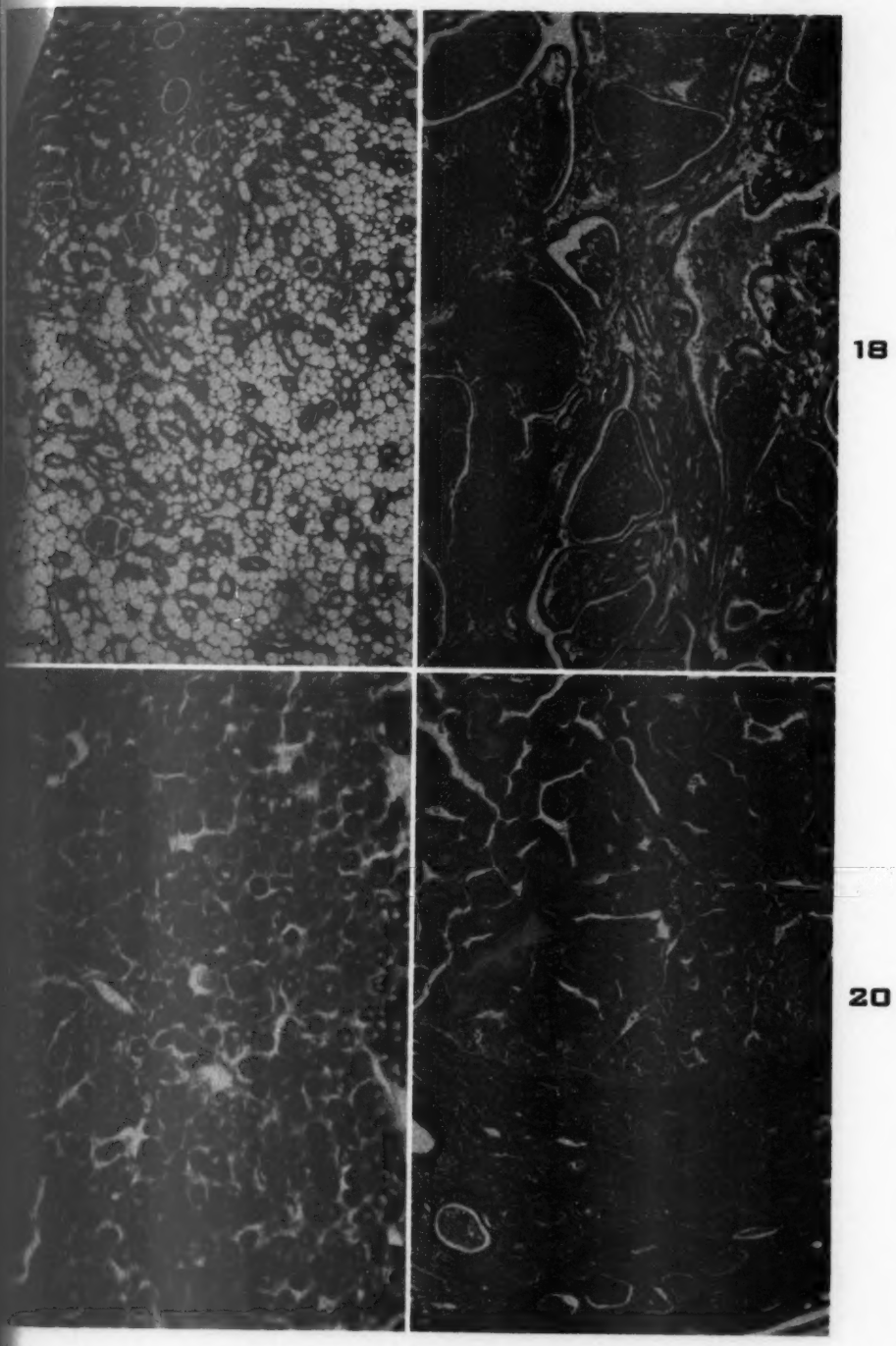

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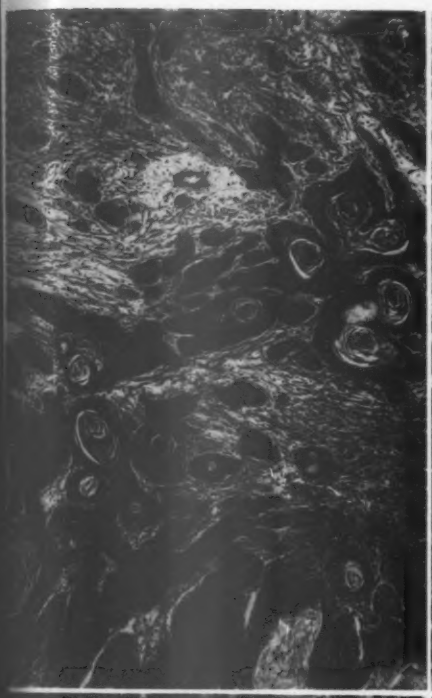
FIG. 21. Squamous cell carcinoma of esophagus. × 60.

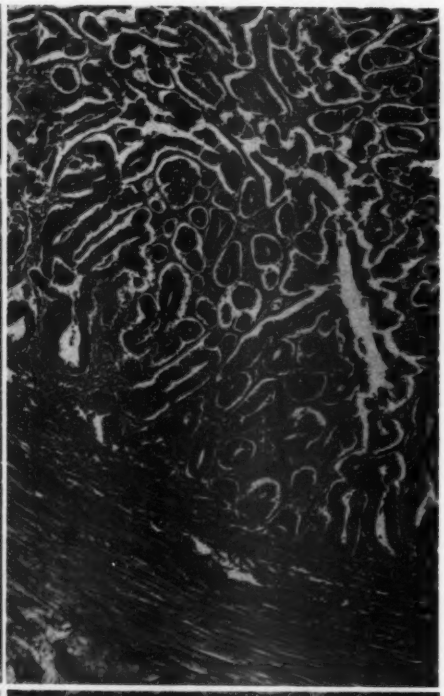
FIG. 22. Adenocarcinoma of colon. × 100.

FIG. 23. Mucoid carcinoma of stomach. × 60.

FIG. 24. Papillary cystadenocarcinoma of ovary. × 60.

VIRTUAL REALITY





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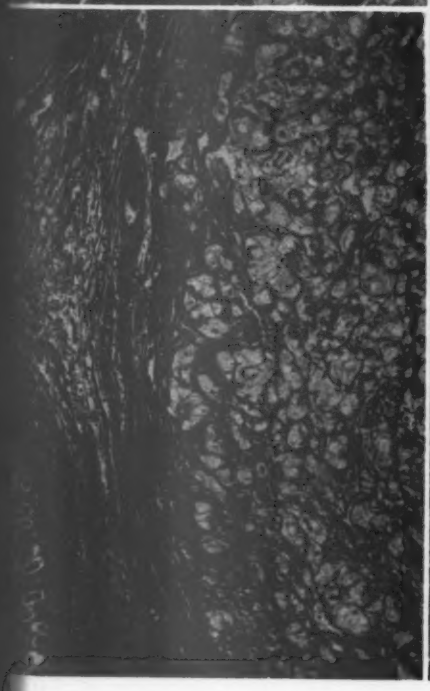

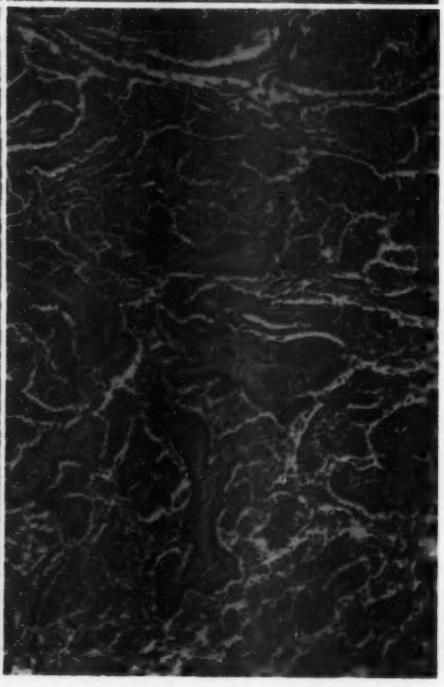

24

PRIMARY CUTANEOUS INOCULATION TUBERCULOSIS IN THE MACACA MULATTA MONKEY*

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Primary cutaneous tuberculosis in monkeys evidently has not been reported in the literature. This paper deals with cutaneous lesions induced in monkeys accidentally inoculated with tuberculous exudate while being tattooed for identification.

Robert Koch[1] demonstrated that the introduction of tubercle bacilli into the skin of the guinea pig led to the development of the primary cutaneous complex of tuberculosis, while subsequent inoculations caused only local lesions which healed early without spread. Kral,[2] in 1953, reviewed the occurrence of cutaneous tuberculosis of cattle, sheep, goats, swine, horses, dogs, cats, and birds. Only two types of lesions were mentioned by name: scrofuloderma of cattle, sheep, and goats, and *tuberculosis verrucosa cutis* in parrots. Circumscribed tuberculous nodules of varying size in the skin or subcutaneous tissues of other species were also described. Kral made no attempt to differentiate between primary and secondary skin tuberculosis in domesticated animals.

Ghon[3,4] described the primary complex of tuberculosis in man. It was named the Ghon complex and defined by Ranke[5] and Krantz[6] as the primary focus of infection at the site of inoculation, with involvement of regional lymph nodes in persons not previously infected with the tubercle bacillus. Bruusgaard[7,8] showed that primary inoculation could occur by the cutaneous route. Inoculation tuberculosis of the skin and the nature of the primary cutaneous complex of tuberculosis in man have been reviewed by Stokes,[9] Krantz,[6] O'Leary and Harrison,[10] and Michelson.[11]

It is often impossible to recognize the primary cutaneous complex in man, because of the difficulty of excluding prior infection. Most of the authentic cases have been described in children, in whom it could be shown that freedom from infection existed at the time of inoculation. Blumenthal[12] characterized primary and subsequent forms of cutaneous tuberculosis as follows:

Primary Inoculation: (1) A papule followed by an ulcer at the

* Received for publication, August 22, 1957.

point of inoculation. (2) Lymphangitis and regional adenitis. (3) Insensitivity to tuberculin at first, positive reaction later.

Subsequent Inoculation: (1) Shorter incubation period. (2) Severe local inflammation. (3) Positive tuberculin reaction from beginning.

Material

Six juvenile *Macaca mulatta* monkeys, 2 males and 4 females, were accidentally inoculated with tuberculous exudate at the time of tattooing identification marks. These animals serve as the basis for this study.

The monkey colonies in this laboratory are confined, 2 monkeys to a cage, in portable, double-deck, steel, dog cage units. Each cage measures 24 by 34 by 26 inches. They are housed in 14 air-conditioned rooms measuring 30 by 30 feet. One hundred monkeys in 25 cage units are kept in each room. There is a clean corridor with a separate door to each room through which new food, bedding and cages are carried. Each room has an additional door leading to a separate refuse corridor through which old food, bedding and cages are removed. Thus, clean and old materials never cross paths.

Each pair of monkeys is fed daily rations of one pound of dry pellet laboratory feed with vitamin C, with one quart of water in an inverted laboratory water bottle.

The monkeys of each new shipment are tuberculin tested on arrival, and every 2 weeks as long as they are kept in the quarantine colony (usually 6 to 8 weeks). The tuberculin test is administered by intradermal injection of 0.1 ml. of a solution of 1 part of Koch's old tuberculin in 19 parts of sterile isotonic saline solution into the right upper eyelid. This site is observed at 24 and 48 hours, edema of the eyelid indicating a positive reaction. Those showing typical positive reactions and their cagemates are always sacrificed and examined at necropsy. Suspected reactors, having too little edema or too intense erythema for diagnosis, are immediately tested again in the left eyelid. Those animals continuing to have doubtful reactions are kept and tested again 2 weeks later along with the entire group.

Each monkey is tattooed for identification on arrival. The chest is clipped with electric animal clippers and the identifying mark of one letter, and 1, 2 or 3 numerical digits is applied in black tattoo ink with an electric tattoo instrument. The tip of the tattoo instrument consists of 4 needles which vibrate, lengthwise, in and out of a tubular casing. A tattoo is applied by dipping this tip into the ink and writing as it vibrates. It is necessary to dip the tip into the ink for every 2 or 3 units written.

History of the Cases

A shipment of 100 *Macaca mulatta* monkeys was received on June 26, 1956. These were given numbers from G 570 to G 669 in numerical sequence. Upon admission, monkey G 590 was found to have a ruptured abscess in the right axillary region, with exudate spread across the chest. Cutaneous abscesses are often found in new shipments of monkeys, but once ruptured they generally heal very satisfactorily. The monkeys were tattooed as usual. Twenty-four hours after the injection of tuberculin, monkey G 590 showed a typical positive reaction. This animal and its cagemate G 591 were immediately sacrificed and examined at necropsy.

The remaining 98 monkeys of this shipment were tuberculin tested again on July 12, 1956, and none had positive reactions. Upon being retested on July 31, 1956, 5 of them (G 593, G 594, G 595, G 597 and G 608) were found to have positive reactions. Their cagemates (G 592, G 600, and G 609) did not react. All were sacrificed, and necropsies were performed on August 2, 1956. Each of the monkeys with positive reaction had an ulcer in its tattoo mark. During the fourth test on August 14, 1956, an ulcer was found on the tattoo mark of monkey G 613. This monkey was immediately sacrificed and examined at necropsy. It had no cagemate. At the fifth test on August 27, 1956, no positive reactions were found.

Of this shipment, all surviving monkeys having no evidence of tuberculosis were later assigned to a research laboratory, where follow-up studies were not undertaken. All of these monkeys were used in the safety test for the Salk vaccine. They were tested once more during the following month, and were sacrificed at the end of that month. None were found to have tuberculosis.

Observations

The general physical condition of monkey G 590, a positive tuberculin reactor, was good. However, an ulcerating lesion was found in the right axillary space which exuded white purulent material when pressure was applied. Of the 4 axillary lymph nodes beneath the ulcer, 2 were enlarged and congested, and a third node contained a small area of caseation. The fourth node was the site of extensive caseation and communicated with the skin ulcer by a sinus tract. The spleen was slightly enlarged and contained numerous caseous tubercles.

Monkeys G 593, G 594, G 595, G 597, G 608 and G 613, all positive reactors to the tuberculin test, were in good general condition. Each had essentially identical lesions in the skin of the chest and in

the axillary lymph nodes. In each case, the skin lesion, located directly on a tattoo mark, was a single, small, inconspicuous, slightly raised, crust-covered ulcer overlying a small pocket of pale yellow pus (Fig. 1). The axillary lymph nodes were enlarged and congested, and one or more nodes of each affected group contained extensive caseation. Three of the monkeys had unilateral adenitis, and 3 were affected bilaterally. No lesions were found in other portions of the carcasses.

No attempt was made to culture micro-organisms from the lesions found.

Sections of the axillary lesion of monkey G 590, when examined microscopically, revealed evidence of tuberculous lymphadenitis and scrofuloderma. The splenic lesions were tuberculous granulomas and appeared to be developing within lymphoid follicles. Numerous acid-fast bacilli were found in the caseous granulomas in the axillary nodes and in the spleen. The bacilli were abundant in the sinus exudate of the scrofulodermatous lesion.

The cutaneous chest lesions of monkeys G 594, G 595, G 597, G 608, and G 613 were also tuberculous. The cutaneous lesion of monkey G 593 was not examined microscopically. Axillary lesions of tuberculous lymphadenitis were observed in each of the 6 monkeys.

The skin lesions showed essentially similar granulomatous reactions. These were suppurative dermal abscesses, each surrounded by a layer of epithelioid cells. At the periphery of the abscess, the dermis and outer third of the subcutis were infiltrated in an irregular fashion by lymphocytes, plasma cells, histiocytes and fibroblasts. Remnants of hair follicles remained in these areas. Secreting cells of sweat glands were swollen and acidophilic. Numerous congested vessels were present around each lesion. The overlying epidermis was necrotic, infiltrated by neutrophils, and compressed layers of it appeared to be detached from the underlying dermis. The epidermis at the edge of the lesion was acanthotic and blended gradually with the adjacent normal epithelium (Fig. 4). The lesions ranged from 3 to 5 mm. in diameter, varying with the intensity of dermal infiltration by granulomatous elements. One lesion was characterized by a single, distinct tubercle circumscribed by a well-organized zone of epithelioid cells (Fig. 2). In the remaining 4 lesions, the form of the tubercles was indistinct due to irregular arrangement and intermingling of the layers of inflammatory cells (Fig. 3). Aggregates of mononuclear leukocytes and epithelioid cells near blood vessels of the dermis and subcutis simulated granulomatous lymphangitis, although the relationship of

the lesions to lymphatic vessels could not be clearly established. A multinucleated giant cell was found in only one of the 5 skin lesions. It was located in a cellular cluster adjacent to a subcutaneous blood vessel.

Twenty axillary lymph nodes from the 6 monkeys were examined. In each instance, caseous granulomas had evidently destroyed one fourth to three fourths of one or more nodes in each affected axillary group. Multinucleated giant cells were occasionally found. The capsules of the lymph nodes were thickened in areas near the tubercles, and the perinodal tissue contained granulomatous elements.

Acid-fast staining bacilli, characteristic of *Mycobacterium tuberculosis*, were observed in each of the 5 cutaneous lesions examined. They were difficult to find in only one of these lesions. Microorganisms were numerous, however, in the affected regional lymph nodes of all 6 of the monkeys. Since cultures were not made, the type of tubercle bacillus present was not determined.

Discussion

The clinical and pathologic observations indicate that the lesions produced in these accidentally inoculated monkeys were those of primary cutaneous tuberculosis, since the monkeys did not react to tuberculin testing at the time of inoculation, and their reaction remained negative until 4 weeks after inoculation. Furthermore, the cutaneous lesions and regional adenitis were the only tuberculous foci found in the monkeys at necropsy.

The chronologic development of the disease in the monkeys should be compared with that in man, especially since the date of inoculation was known. Montgomery[13] reported that the tuberculin reaction in man usually became positive 3 to 6 weeks following the onset, and that extension to the regional lymph nodes occurred at about the same time. Michelson[11] stated: "The glands show a reaction in from three to four weeks after injury, although it is hard to fix the time of inoculation with certainty." In comparison, 5 of the monkeys also showed positive reactions at some time between the second and fourth weeks after inoculation. Regional adenitis with caseation necrosis was present in all monkeys sacrificed at 4 weeks.

According to Montgomery,[14] the initial lesion is histologically nonspecific and is characterized by ulceration, infiltration by polymorphonuclear leukocytes, lymphocytes, plasma cells, and by varying degrees of necrosis. He stated also: "Within from three to six weeks after the onset, and at about the same time that there is extension along

the lymphatics to adjacent lymph nodes, the histologic picture changes to that of specific tubercle formation with increasing numbers of epithelioid cells and early formation of giant cells; as this occurs the tubercle bacilli become fewer and increasingly difficult to demonstrate." He noted that inoculation tuberculosis in its fully developed phase is characterized by specific tubercle formation and a marked degree of caseation necrosis. Similarly, the monkey lesions at 4 weeks were granulomatous abscesses resembling tubercles. The tubercles, however, were often difficult to delineate. One lesion observed 6 weeks after inoculation was no more advanced in tubercle formation than those observed at 4 weeks. Giant cells were conspicuously absent from the cutaneous lesions, and necrosis had not reached the stage of true caseation. In only one cutaneous lesion was it difficult to find acid-fast staining bacilli.

Primary cutaneous tuberculosis apparently progresses at about the same rate in monkeys as in man. Assuming that the lesion in monkeys attains the same development as that in man,[14] the lesions described in this paper evidently were not fully developed at 4 to 6 weeks following inoculation.

The lesions differed primarily from reinfection tuberculosis in having regional adenitis and limited invasion of tissue. Although the epidermis was consistently acanthotic, verrucose hyperplasia was not evident. The monkeys revealed more extensive areas of abscess formation than is usually found in lupus vulgaris.

Summary

Six juvenile *Macaca mulatta* monkeys were accidentally inoculated intradermally with tuberculous exudate which had contaminated the needles of a tattooing machine.

Clinical and histologic observations indicated that the lesions produced in the monkeys were those of the primary cutaneous complex of tuberculosis.

The chronologic development of the lesions was found to be similar to that of the primary cutaneous complex of tuberculosis in man.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Cutaneous tuberculous lesion of the chest of monkey G 613, showing the inconspicuous appearance of the ulcer and its position on a tattoo marking.

FIG. 2. The cutaneous lesion of monkey G 608, showing relatively advanced tubercle formation. Hematoxylin and eosin stain. × 26.

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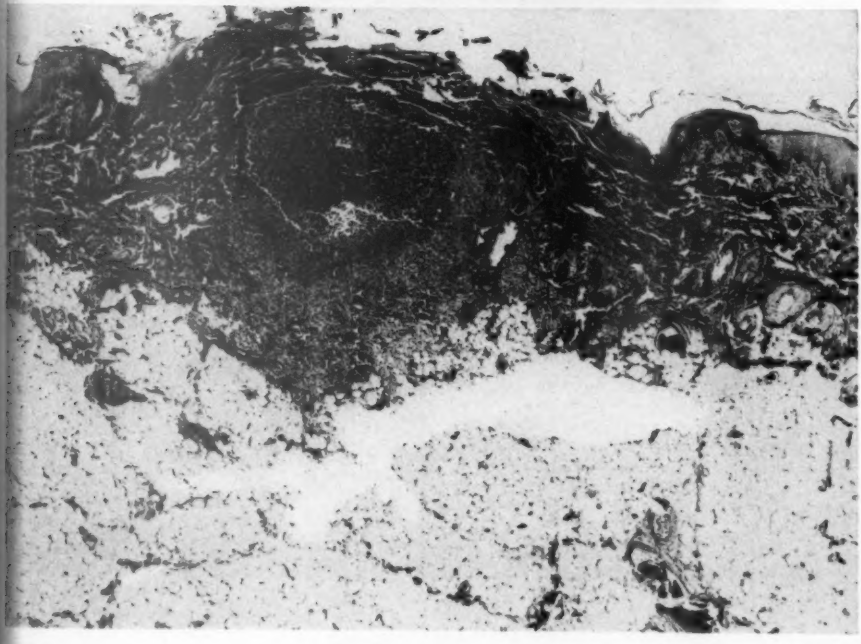

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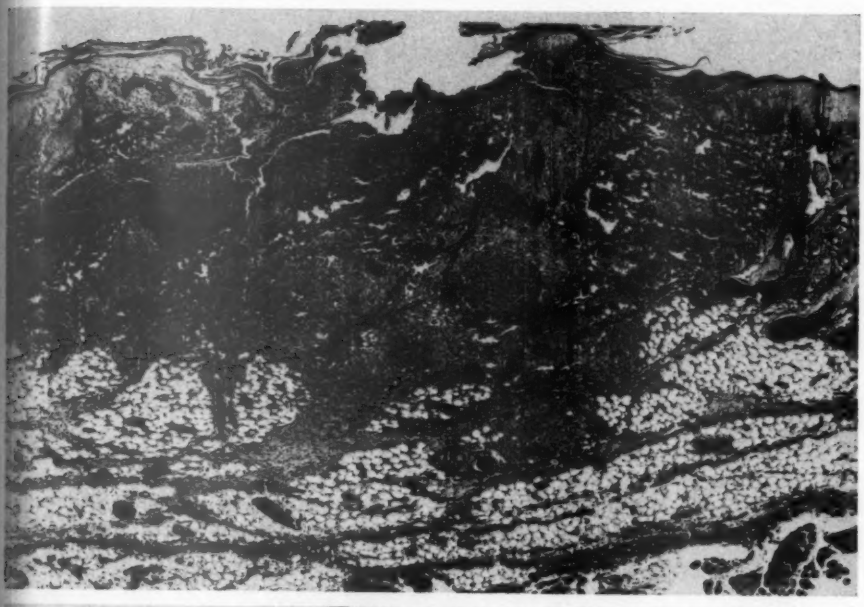
FIG. 3. The cutaneous lesion of monkey G 594, showing a granulomatous abscess with early tubercle formation. Hematoxylin and eosin stain. × 26.

FIG. 4. The cutaneous lesion of monkey G 597. The area sectioned was adjacent to the ulcer and shows acanthosis of the epidermis overlying the abscess. Hematoxylin and eosin stain. × 25.



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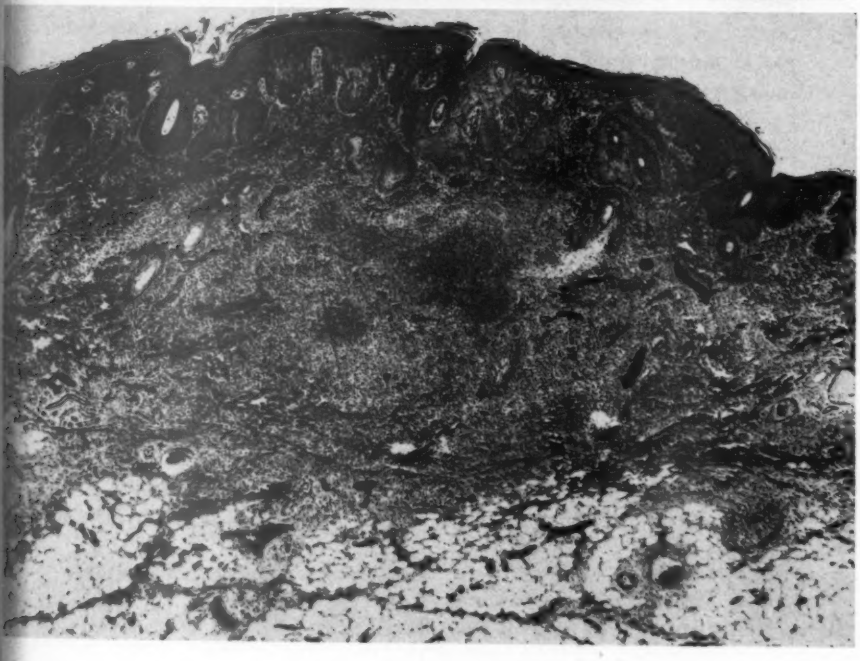

4

ALTERATIONS IN THE DORSAL ROOT GANGLIA AND ADJACENT NERVES IN THE LEUKEMIAS, THE LYMPHOMAS AND MULTIPLE MYELOMA*

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Almost 130 years have passed since Gietl[1] suggested that changes might be present in the spinal ganglia in various fevers and that these changes might be the cause of St. Vitus' dance and other diseases. In the ensuing period there have been isolated studies concerning changes in dorsal root ganglia with aging and disease.[2-6] Few of these, however, have been comprehensive or adequately controlled. The present investigation, a portion of a general evaluation of the dorsal root ganglia at necropsy, is limited to a study of the dorsal root ganglia in patients with leukemia, malignant lymphoma and multiple myeloma.

MATERIAL AND METHODS

The dorsal root ganglia collected during a 4½-year period were procured from two departments of pathology. At the City of Detroit Receiving Hospital, an emergency hospital, there were 3,234 patients necropsied; from 2,129 of these at least one ganglion and attached nerve was obtained. This series included 68 patients with leukemia, lymphoma or multiple myeloma. During the same period 1,355 necropsy examinations were performed at the Veterans Administration Hospital in Dearborn, Michigan; among these there were 88 patients with the diseases noted above. Suitable tissue from dorsal root ganglia and attached peripheral nerves was available for study from both hospitals in 92 of the 156 patients with the diseases under investigation.

Because of the method utilized for removal of the spinal cord, the ganglia were invariably procured from the right midlumbar region. Occasional ganglia were considered to be inadequate in size or improperly fixed and not suitable for study. In most instances the specimen consisted of a portion of peripheral nerve, the dorsal root ganglion, the central nerve root, and the surrounding dura and supporting tissues.

All material was fixed in 10 per cent formalin, embedded in tissuemat or paraffin, and sectioned at 7 μ. Ten sections stained with

* Received for publication, July 5, 1957.

hematoxylin and eosin and one section stained with luxol fast blue silver nitrate for myelin and axis cylinders were made in each case. Cresyl violet stains for amyloid were prepared in all cases of myeloma. Supplementary stains included Masson's trichrome, Prussian blue for iron, Mallory's phosphotungstic acid hematoxylin, and Mahon's stain for myelin. The investigation encompassed all clinically and histologically proven cases of leukemia, all types of lymphoma except those localized to one area of the body, and all patients with multiple myeloma. These were selected and confirmed by a review of necropsy reports and by microscopic examination of the necropsy material.

The sections of the dorsal root ganglia with their nerves and perineural tissues were examined with special attention being given to the character, the activity, and the degree of change in the ganglion cells. The location and the amount of fibrosis and Schwann cell proliferation (Fig. 1), the alterations in myelin, axis cylinders (Fig. 2), nerve sheaths and blood vessels were also investigated. The presence of cellular infiltration was described with specification of location, nature and extent. When possible, alterations were graded on a basis of three degrees: minimal (1+), moderate (2+), and marked (3+). Observations made independently were later compared and differences resolved by re-evaluation.

The control for this portion of the study consisted of a similar examination of dorsal root ganglia procured from the other necropsies at the City of Detroit Receiving Hospital.

Observations

Malignant Lymphoma

Müller's inaugural dissertation is the earliest report that discusses the involvement of the peripheral nervous system in leukemia.[7] In this, Müller suggested that leukemic involvement of peripheral nerves had resulted in a peripheral neuritis; however, there was no histologic proof provided. Involvement of the peripheral nervous system by lymphomatous infiltration exclusive of the cranial nerves has been reported in only 6 histologically proven cases, in 4 of which the spinal ganglia were also affected.[8-13] In another patient, degeneration of the ganglion and peripheral nerve was considered to be unrelated to lymphoma.[14] From the paucity of these reports, it is apparent that the peripheral nervous system is either rarely involved or rarely examined in patients with any of the forms of this disorder. Clinical studies suggest that the latter is more likely.[12,15-17]

The peripheral nervous system was examined at necropsy in 50 patients with malignant lymphoma. Included in this group were 30

patients with Hodgkin's disease, 7 with reticulum cell sarcoma, 4 with follicular lymphoma, 9 with lymphosarcoma, 9 with chronic lymphocytic leukemia and one each with acute and subacute lymphocytic leukemia.

Dorsal root ganglia were available for study in 29 of the 30 patients with Hodgkin's disease. Only the peripheral nerve was satisfactory for study in the remaining patient. The degenerative changes in the ganglia were mild in 14, moderate in 12, and severe in 3. There was no significant abnormality in the attached peripheral nerve in 2. A mild peripheral neuropathy was found in 21, while moderate changes were noted in the sections from 7 patients. There were no cellular infiltrations in 10 of the 30 cases. In 10 there were one or more lymphocytic infiltrations: in the central nerve root in 3, the dorsal root ganglion in 4, the peripheral nerve in 5, and the epidural tissues in one. A mixed lymphocytic and monocytic infiltration was seen in 2 instances, once in the dorsal root ganglion and once in the epidural tissues. In 5 cases the infiltrations were composed of "monocytic" and reticulum cells; these appeared 4 times in the central nerve root and once in the dorsal root ganglion. On 3 occasions the peripheral nerve was the seat of this process and in one, the epidural tissue. An infiltration considered indicative of Hodgkin's disease was found 3 times in the epidural tissue but never in the nerve or spinal ganglia. In two instances perineural phlebothrombosis was found.

Spinal ganglia and attached nerves were removed in 6 and a peripheral nerve alone in one of the 7 cases of reticulum cell sarcoma. Degenerative changes in the ganglia were mild in one case and moderate in 5. The degree of neuropathy was mild in all. A focal lymphocytic infiltration was present in one peripheral nerve. In two cases there was a mixed lymphocytic and monocytic infiltration; this occurred once in the central nerve root and once in the peripheral nerve. The latter lesion was that of peripheral neuritis (Fig. 3). Reticulum cells comprised the infiltration in 3 patients; 3 times in the epidural fat, once in a central nerve root and once in a dorsal root ganglion. In one specimen there was no infiltration.

There was a mild degenerative change in the dorsal root ganglion cells in one and moderate change in 3 of the 4 patients with follicular lymphoma. The degree of neuropathy was mild in 2 and moderate in 2 cases. Two proved to be free of neoplastic infiltration. A nodular lymphocytic infiltration was found in the ganglion of one patient (Figs. 4 and 5) and diffuse infiltration in and about the central nerve root in the other (Figs. 6 and 7). A definite peripheral neuritis was found in one case.

From 9 patients with lymphosarcoma 7 specimens included dorsal root ganglia and nerves, and 2 the peripheral nerve alone. Degenerative changes in the ganglia were mild in 5, moderate in one and severe in one. There was no neuropathy in 4, mild neuropathy in 2, moderate in 2, and severe in one. There were lymphocytic infiltrations in 7 instances. These were seen in the dorsal root ganglia of 4, central nerve roots of 2, in the peripheral nerves in 5, and 3 times in the epidural fat. The lesions were not considered diagnostic of lymphoma. In 2 specimens there were no evidences of infiltration.

In the 9 patients with chronic lymphocytic leukemia, there were 8 from whom adequate specimens of dorsal root ganglia and attached nerves were obtained and one with a peripheral nerve only. Mild degenerative changes were found in the ganglia in 5 cases, while in 2 the changes were moderate, and in one of severe degree. The latter lesion was associated with a peripheral neuritis. There was a mild neuropathy in 6 instances and moderate alteration in 2. In 4 specimens there was no cellular infiltration. In the 5 remaining cases a nonspecific lymphocytic infiltration was seen in 3 ganglia, in 4 peripheral nerves, in 2 central nerve roots and in 2 sections of epidural tissue.

Dorsal root ganglia were available for study in each of the two patients with acute and subacute lymphocytic leukemia. A mild degenerative change in the ganglia and a mild neuropathy were noted in both. Cellular infiltrations, present in each case, were considered as indicative of leukemia. They were present in the dorsal root ganglion and epidural tissues of one, and in the central nerve root of the other. In the former, there was also hemorrhage into the dorsal root ganglion, while in the latter there were multiple venous and capillary thrombi.

Leukemia

Following Müller's dissertation[7] less than 10 (6 plus "several"[21]) histologically proven examples of leukemic involvement of the peripheral nerves[16,18-21] were reported, and there have been 4 or possibly 5 additional instances in which lesions of dorsal root ganglia were noted.[20-23] Clinical studies suggest that leukemic lesions in these areas occur far more commonly than one would gather from pathologic reports.[2,16,24]

The forms of leukemia in the 22 patients of this group have been designated in the following manner: 3 cases of leukemic reticulosis, 3 of stem cell leukemia, 4 of monocytic leukemia, and 12 of myelogenous leukemia.

The dorsal root ganglia were examined in 2 and a peripheral nerve in another of the 3 patients with leukemic reticulosis. The degenera-

tive changes in the ganglia were mild in one and moderate in the other. No significant neuropathy was found in any of the 3.

Moderate degenerative changes and a mild neuropathy were present in the dorsal root ganglia and adjacent nerves in all 3 cases with stem cell leukemia. There were neoplastic but nondiagnostic infiltrations in 2 of the ganglia and in one peripheral nerve. Peripheral neuritis was found in one case.

Ganglia were procured from 4 patients with monocytic leukemia (acute, subacute and chronic forms). Degenerative changes in the ganglia were more severe than expected in one patient. In 3 there were cellular infiltrations, only one of which was considered leukemic. In one, recent hemorrhage was encountered in the central nerve root and in the ganglion. Nonspecific infiltration with lymphocytes, plasma cells and monocytes was found in the peripheral nerves of 2 cases, the nerve roots of 3, and in the epidural tissues of 2.

Among the 12 patients with myelogenous leukemia, adequate dorsal root ganglia and attached nerves were procured from 9 and peripheral nerves alone from 3. Degenerative changes were marked in 6 of the ganglia and neuropathy of moderate degree was found in 2 specimens. In 8 cases there were recognizable leukemic infiltrations; 3 appeared in the dorsal root ganglia (Figs. 8 and 9), 3 in peripheral nerves, 2 in central nerve roots and 8 in the epidural tissues. Nonspecific perivascular infiltrations with lymphocytes were noted in one patient. Additional findings included peripheral neuritis in one patient, perineural venous thrombosis in 2, and hemorrhage in both the peripheral nerve and ganglion in one.

Multiple Myeloma

An early report of peripheral nerve abnormality in multiple myeloma is that of Davison and Balser[25] which records 6 patients in whom the peripheral nervous system was affected by compression. There have been several more recent reports[26-31] but in none are myelomatous infiltrations of nerves or ganglia noted.

In the present study there were 9 cases of multiple myeloma; from 8, dorsal root ganglia and nerves were procured and from one, a peripheral nerve only. Degenerative changes in the ganglia and peripheral nerve were pronounced in 5 cases. Nonspecific infiltrations, composed of lymphocytes, monocytes and plasma cells, were found in the dorsal root ganglia of 3 cases, the peripheral nerves of 3, the central nerve roots of one and the epidural tissue of 3. There were no infiltrations in 3 patients. In one nerve there was a recent hemorrhage and in 2 a peripheral neuritis. A nodular deposit of

amyloid in the fat adjacent to the peripheral nerve was seen in one case. Stains for amyloid were negative in the nerves and ganglia of all. Neoplastic infiltrations were not encountered in any of the myeloma cases.

Discussion

Estimates of the incidence of clinically detectable peripheral neuritis in patients with malignant lymphoma have varied from 15 to 36 per cent.[15,17] A similar incidence has been reported in patients with leukemia and multiple myeloma.[18,22,25,27] It has been suggested that the peripheral neuritis is the result of neoplastic infiltration of the nerves, that it is of toxic origin, the result of compression of nerve roots by pathologic fractures of the vertebrae or by epidural tumor masses.[9,18,22,27,28,30,32-34] A neuropathy due to amyloidosis has also been postulated in multiple myeloma.[35]

In the present study cellular infiltrations in and around peripheral nerves, central nerve roots and the spinal ganglia were present in 66 of the 92 cases surveyed. These were of nonspecific nature and presumably inflammatory in 39; they simulated the underlying neoplastic lesion in 11, and were frankly identifiable as neoplastic in 16. In all of the patients with multiple myeloma and in all but two with Hodgkin's disease, the process was a nonspecific one. Eight lesions of nonspecific character were associated with active peripheral neuritis. In the remaining 31 cases the alterations formed part of a less severe inflammatory process that involved the nerves and ganglia.

It should be emphasized that the lesions observed in this study did not indicate the extent of neural involvement; there were rarely more than a few sections of one ganglion and nerve examined, and specimens were procured without regard to clinical findings. Moreover, the samples utilized probably represented the portions of the nervous system last altered in patients with a peripheral neuropathy.[36] The degenerative changes noted were not significantly greater than those encountered in patients dying of other causes.[2,4]

The histologic patterns of the cellular infiltrations recognizable as of neoplastic nature could be divided into two types, each with approximately equal frequency. In the first the infiltrations tended to be diffuse but with a slight perivascular predominance, while in the second the lesions were focal and compact. The cellular aggregates were most frequently present in the tissues about the dorsal root ganglia and attached nerves; next in frequency was the involvement of peripheral nerves, and least often diseased were the central nerve roots and dorsal root ganglia. It is interesting that cellular infiltra-

tions, even those within the nervous system did not regularly cause recognizable injury. They might, however, have been the cause of transient neurologic signs and symptoms.[37]

The reasons for the appearance of nonspecific, probably inflammatory, infiltrations is not known. Several investigators have suggested the existence of nutritional deficiencies in these patients; others have proposed as causes, the action of toxins or the alterations in cell metabolism that may accompany these disorders.[2,3,38,39] Three other possible contributory factors can be added; namely, the action of therapeutic agents utilized, the effects of latent or acquired infections[40] or the compressive effects of adjacent neoplastic infiltrations.[41]

The high incidence of hemorrhage encountered was undoubtedly representative of the bleeding tendencies common in these diseases. The incidence of venous thrombosis is comparable to that in other patients with chronic debilitating disorders.

Summary

1. Significant cellular infiltrations were found in the dorsal root ganglia and attached nerves and tissues in 66 of 92 patients with malignant lymphoma, leukemia and multiple myeloma examined at necropsy. The lesions were considered to be related to the neoplastic process in 16 patients, equivocally related in 11, and unrelated (nonspecific and inflammatory) in 39 individuals.

2. Lesions of the peripheral nervous system, including the dorsal root ganglia, occurred with a frequency far greater than might have been anticipated by the clinical manifestations.

3. Primary or secondary lesions of the peripheral nervous system should be suspected in patients with neoplastic diseases of the hematopoietic system.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

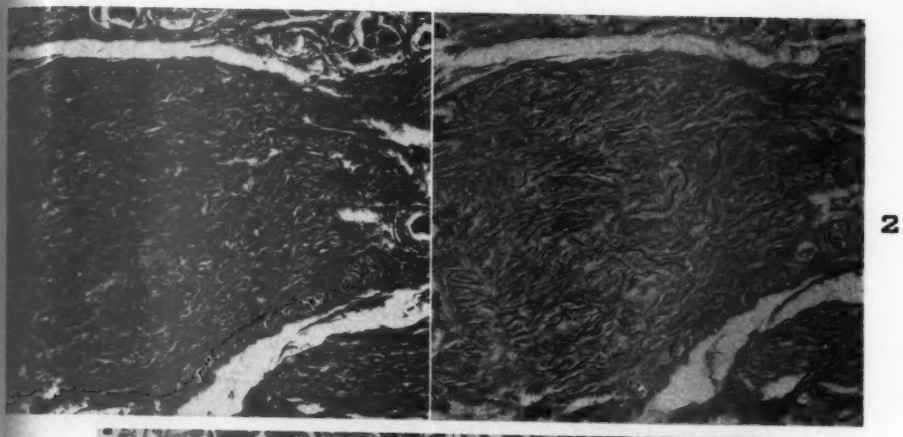
FIG. 1. Marked peripheral neuropathy with Schwann cell proliferation. Hematoxylin and eosin stain. × 90.

FIG. 2. Marked focal swelling of axis cylinders of peripheral nerve. Luxol fast blue silver nitrate stain. × 90.

FIG. 3. Chronic peripheral neuritis in a case of reticulum cell sarcoma. Hematoxylin and eosin stain. × 90.

FIG. 4. Nodular lymphocytic infiltration in dorsal root ganglion of patient with follicular lymphoma. Hematoxylin and eosin stain. × 90.

FIG. 5. A higher power view of the lesion shown in Fig. 4. The lymphocytes exhibit no attempt at follicle formation. Hematoxylin and eosin stain. × 280.



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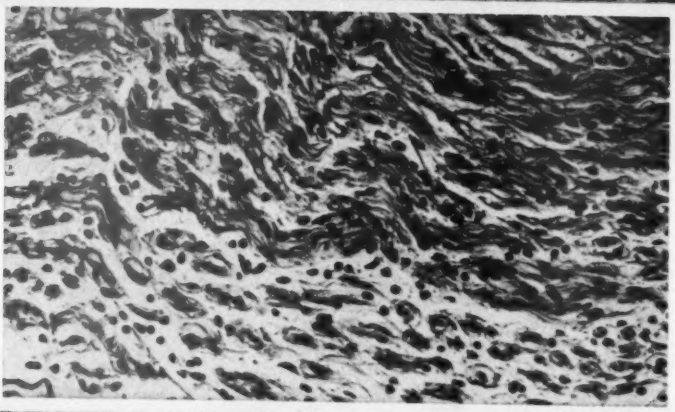

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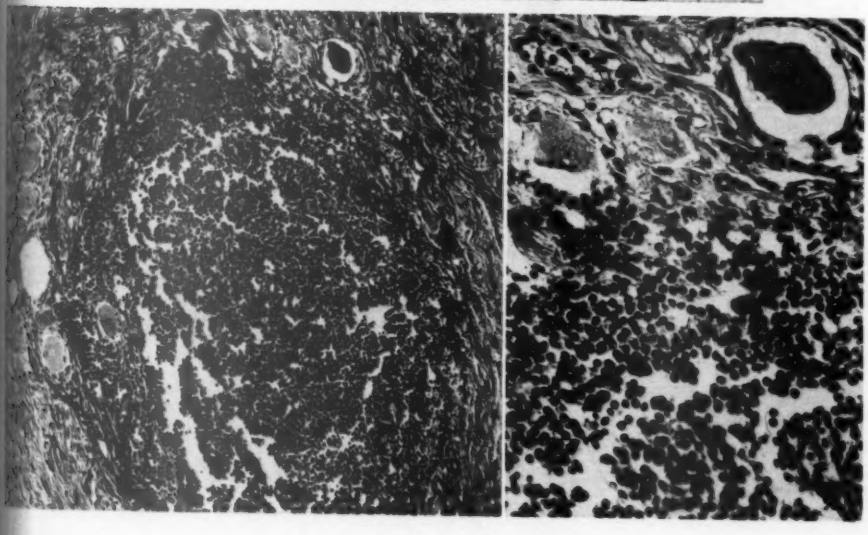

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FIG. 6. Diffuse cellular infiltration in a central nerve rootlet in a case of follicular lymphoma. Hematoxylin and eosin stain. × 90.

FIG. 7. A higher magnification of the lesion shown in Fig. 6. The infiltration is composed largely of mature lymphocytes with no attempt at follicle formation. Hematoxylin and eosin stain. × 280.

FIG. 8. Diffuse leukemic infiltration and moderate degenerative change in dorsal root ganglion of patient with acute myelogenous leukemia. Hematoxylin and eosin stain. × 90.

FIG. 9. Higher magnification of the lesion shown in Fig. 8. The cells tend to separate and destroy the components of the ganglion. Hematoxylin and eosin stain. × 280.

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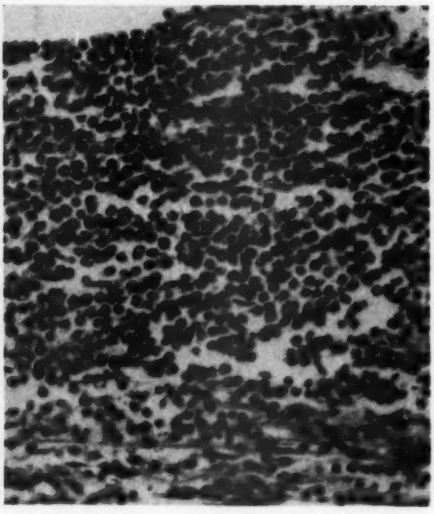

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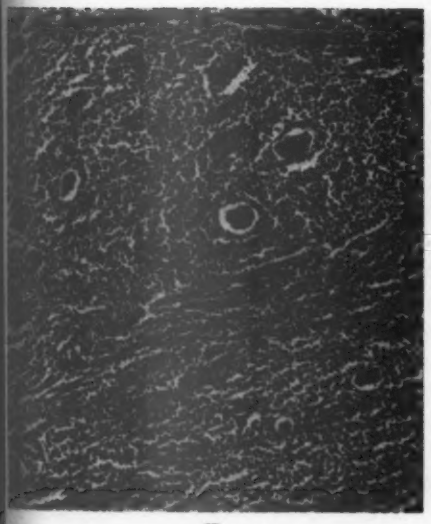

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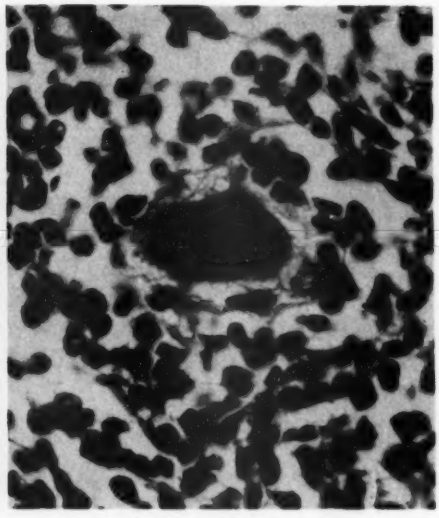

9

THE GROUND SUBSTANCE OF THE CENTRAL NERVOUS SYSTEM IN MAN*

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The past few years have produced a number of papers concerning what has been defined as the "ground substance of the central nervous system." In particular, Hess,[1-4] using certain histochemical methods, has described a ground substance observed in the gray matter of the rat, mouse, and guinea pig, and has related its appearance and presence to the blood-brain barrier. Other workers[5-7] have mentioned the ground substance of the brain in relation to various pathologic processes in man without, however, offering a histologic demonstration of it. Still others[8] have sought to study it by means of electron microscopy.

The present investigation was undertaken in an attempt to determine whether features comparable to those reported by Hess in the rat,[1] mouse[1,3] and guinea pig[2] could be demonstrated in human material and, if so, what their relationship to the clinical problem of the blood-brain barrier might be.

MATERIAL AND METHODS

The material used in this study consisted of 18 specimens comprising the complete central nervous system of human embryos, fetuses, infants, children and adults, ranging in age from 8 weeks of intra-uterine life to adulthood.

The fetuses were obtained from surgically induced abortions and were sent to us fixed in Bouin's fluid through the courtesy of the Wenner Gren Cardiovascular Research Laboratory and from the Obstetric Clinic of St. Erik's Hospital, Stockholm, Sweden.‡ In each, detailed data concerning the duration of pregnancy, as well as the weight of the complete fetus, were carefully recorded. The specimens from neonates, infants, children and adults were obtained from patients at the Flower and Fifth Avenue Hospitals, who died of diseases

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not affecting the central nervous system, either directly or indirectly (Table I).

From these specimens, multiple sections of the cerebral cortex, basal nuclei, cerebellum, pons, medulla oblongata and spinal cord were prepared. Additional tissue was studied from cases in which only segments of the central nervous system were available for suitable investigation. These were procured from 22 subjects of varied age (Table I).

TABLE I

Age Range of Cases From Which Specimens of the Central Nervous System Were Obtained

Age	No. of cases: CNS complete	CNS segments
Prenatal:		
8 to 12 weeks	1	
13 to 24 weeks	5	9
25 to 40 weeks	3	4
Postnatal:		
0 to 3 months	3	4
4 to 12 months	2	3
1 to 3 years	2	1
Adults	2	1

The tissue was fixed in Bouin's solution (picric acid, saturated aqueous solution, 75 ml.; formaldehyde, 25 ml.; acetic acid, 5 ml.) and alcohol-formol (alcohol 95 per cent, 90 ml.; formaldehyde, 10 ml.) and embedded in paraffin in the usual manner.

Sections from each block were cut at thicknesses varying from 4 to 12 μ, attached to the slides by means of distilled water, thus avoiding the use of glycerin albumin, and stained with the following procedures: (1) hematoxylin and eosin; (2) periodic acid-Schiff method with alcoholic solution of periodic acid (PAS-A); (3) periodic acid-Schiff method with aqueous solution of periodic acid (PAS-B) as used in the central nervous system by Wislocki and Singer[9] and later by Hess;[1-4] (4) Ritter and Oleson's method,[10] a combination of the Hale technique for the staining of acid mucopolysaccharides and the PAS-A procedure.

The PAS-B method was also applied after acetylation of some of the sections according to the procedure suggested by McManus and Cason.[11] On selected sections, digestion with testicular and pneumococcal hyaluronidases* was carried out and followed by the Ritter and Oleson staining procedure.

RESULTS

No substantial differences in staining were noted between tissues fixed in Bouin's solution and those fixed in alcohol-formol.

*The testicular hyaluronidase (1,000 TRU per mg.) and the pneumococcal hyaluronidase (450 TRU per mg.) were given to us by Dr. Karl Meyer, College of Physicians and Surgeons, Columbia University, New York, N.Y.

By using the PAS-A procedure, we noticed that the cytoplasm of the neurons and the glial cells remained unstained except for occasional elements. The nuclei and nucleoli exhibited a very faint, nearly imperceptible, positive reaction. The axons remained almost unstained, while the dendrites and processes of the glial cells stained light pink in color.

The connective tissue fibers of the blood vessels gave an intense positive reaction, while in the lumens, the red blood cells were unstained. In addition, we observed that there was a suggestion of a red staining amorphous substance between the connective tissue fibers of the blood vessels. When the PAS procedure was preceded by acetylation, the fibers and this amorphous substance of the vascular wall remained unstained, and when the PAS method was applied after acetylation, followed by treatment of the sections with 0.1N solution of potassium hydroxide, the usual staining was restored.

The meninges and the choroid plexuses showed a marked positive reaction to the PAS stain. The perineurium and endoneural fibrils of the cranial nerves and of the spinal nerve roots were stained a bright red color, as in the case of connective tissue fibers.

With the PAS-B technique, which differed from the PAS-A in the use of an aqueous instead of an alcoholic solution of periodic acid and in omitting the reducing rinse after treatment of the sections with periodic acid, no very significant differences, except for a generally darker staining of the sections, were noted. This darker staining was probably related to iodates and periodates carried over from the oxidizing solution, particularly by combining with metals ($Ca,^{++}$ K^{+}). The retention of the metallic ions has been attributed by Hotchkiss[12] to the omission of the reducing rinse. In view of this, we felt that in our material the PAS-A technique was more reliable.

Acetylation prevented the staining of the sections with PAS, but the positive reaction to PAS reappeared when acetylation was followed by treatment of the sections with 0.1N potassium hydroxide. No satisfactory demonstration of a ground substance was ever noted in the gray matter of any segment of the central nervous system after staining with either PAS-A or PAS-B.

In young embryos (8 weeks gestation), the cells of the inner and middle layers of the various segments of the neural tube were recognized by their very lightly stained nuclei. Cellular processes, stained light pink, were seen to extend through the middle layer into the outer layer, where they formed a rather loose network. No PAS-positive substance was observed between the cells or their processes (Fig. 1). At this stage, no true glial cells were present; it has been

established by Penfield[13] that astrocytes appear in human embryos at about the twelfth week of intra-uterine life.

In later stages of development (Fig. 2), as the architectural organization of the white and gray matter of the various segments of the central nervous system progressed toward the definitive pattern, the number of cellular processes which stained positively with the PAS reaction increased, conferring an over-all darker pink color to the section. In these instances, up to full term fetuses, the gray matter, however, still had a fairly loose architecture, the neuropil appearing as a meshwork of fibrillar processes of various thickness, among which no PAS-positive amorphous substance was seen.

In the newborn (Fig. 3) and in infants, the PAS-positive neuropil becomes gradually more and more compact and eventually, in children under one year of age, reaches the compactness of the nervous tissue of older children (Fig. 4) and adults. In our material, the compactness of the neuropil in children's and adult's brains showed little or no difference. Also, in the latter age groups, evidence of the existence of a PAS-positive substance between cells and tightly arranged cellular processes was not convincing in either the thin or thick sections.

With the Ritter and Oleson technique, nuclei, nucleoli, cellular processes of neurons and supportive glial cells appeared lightly blue stained, while the cytoplasm remained unstained. As with the PAS procedures, no satisfactory evidence of a ground substance of the nervous tissue stained red or blue was observed in any segment of the central nervous system of embryos, fetuses, newborns, infants, children or adults. Within the gray and white matter, only the connective tissue fibers of blood vessels and capillaries were stained bright red. Between these fibers, an amorphous material stained red and blue was observed. This suggested the existence of a ground substance comparable to that found in the blood vessels of other organs by Ritter and Oleson[10] and by Ozzello.[14] In an attempt to establish the nature of the blue component of the vascular ground substance, digestion with testicular and pneumococcal hyaluronidase was carried out and followed by the Ritter and Oleson staining method. No Hale stain positive material was noted after the enzymatic action.

The ground substance encountered between the connective tissue fibers of the blood vessels and, less clearly, of the capillaries, was visible as early as the embryo of 8 weeks gestation (the earliest examined by us), and appeared to become more prominent in later stages of development up to the time of birth. After this time it

showed approximately the same intensity of staining at the various ages.

Comments

The possible existence of a ground substance of the central nervous system and its distribution have proved to be of considerable interest. This is not only the case from a purely histologic standpoint but also—and particularly—from a physiopathologic basis since it has been related to various pathologic manifestations.

The earliest noteworthy mention found in the literature, of a ground substance of the nervous tissue is by Taft,[5] who examined brains of rats and human subjects with the dark field microscope. He felt that there is an interstitial or ground substance in the brain, "the physical and physiologic characteristics of which are analogous in part to those of the serum proteins." This acts as a "purveyor of tissue fluid to and from the brain cells," controlling the osmotic pressures and furnishing at the same time an embedding material for the neurons. He stated that this substance is normally decreased in amount in the newborn and the aged. He also expressed the opinion that the ground substance is altered in pathologic conditions, notably in *dementia paralytica* and perhaps in scurvy.

Bouton[15] related the existence of the blood-brain barrier to what he defined as "intercellular substance" in view of the observation that in the dog the destruction of this substance, as he thought, by air embolism permitted the staining of the nervous tissue by trypan blue.

Hess[1-4] described a PAS-positive amorphous substance between the cells and cell processes of the brain of the rat, mouse and guinea pig, and stated that this ground substance appeared during intrauterine life in the guinea pig and in the newborn mouse, increasing in amount thereafter. With the appearance of the ground substance, the blood-brain barrier became operative. He pointed out that in the guinea pig the appearance of the ground substance of the central nervous system was concomitant with an increase in number and size of the nerve cell processes; i.e., about the 41st to 45th day of fetal life. By using various histochemical procedures, Hess came to the conclusion that the ground substance of the brain was probably a neutral mucopolysaccharide. He concluded that "the ground substance of the central nervous system contributes to the constitution of the blood-brain barrier and is the substance responsible for the ability of normal adult brain to resist vital staining by trypan blue."

It must be pointed out that Wislocki and Singer,[9] using the PAS-B technique, which later was employed by Hess in the above mentioned

investigations, studied cerebral cortex, cerebellum and peripheral nerves, but did not mention any ground substance in their observations. A PAS-positive reaction was also obtained by Massari and Marsico[16] in the central nervous system of various mammals and rodents, but not in the human. These authors noted that there were considerable variations in the degree of positive reaction to the PAS method in the central nervous system among different animals and that it was difficult to establish with certainty which structures were actually stained, largely because of the possible effect of artifacts. Similar results were reported in amphibia by Bairati and Tripoli,[17] who stated that it was premature to express a definite judgment in this matter in view of the limitations of available histochemical techniques.

The isolation of N-acetylhexosamine in cerebral matter as an expression of mucopolysaccharides, was accomplished by Benassi[18] after removal of blood vessels by means of digestion with hyaluronidases (testicular, bacterial, and from *Bothrops jararaca*). He reported isolation of larger amounts of N-acetylhexosamine with the use of testicular hyaluronidase and felt that his results were suggestive of the presence of a ground substance in the central nervous system.

Recently, Dempsey and Wislocki[8] studied the central nervous system of 3 albino rats and one mouse by electron microscopy and expressed the opinion that between the glial processes there existed a very narrow space containing an amorphous substance of moderate electron density. This was in continuation with the substance underlying the capillary endothelium and separating it from the glial endfeet. They further suggested that the "thin lamina of interstitial material . . . differs from the connective tissue ground-substance in that it does not attract silver deposits, and that it is miscible with the connective tissue ground-substance in at least some locations."

Eayrs,[6] studying the changes in the cerebral cortex of hypothyroid rats, cited unspecified alterations in the ground substance as one of the possible causes of lesser density of the neuropil in these animals. He did not, however, give any proof of the existence of a ground substance, but mentioned it as a matter of fact. Similarly, Perret and Kernohan,[7] studying the pathologic changes in the brain caused by space-occupying lesions, particularly tumors, stated that in these conditions "edema is more prominent in the white matter" where "the most prominent change is always seen in the general appearance of the ground substance." They did not, however, provide convincing evidence of the existence of ground substance itself.

We were unable to see any amorphous substance or suggestion of

it in our material which could convince us of the presence of a true ground substance in the tissue of the central nervous system of man stainable with the periodic acid-Schiff reactions. Hess[1-4] made his observations on sections of 15 μ thickness. Our sections were cut at various thicknesses varying from 4 μ to 12 μ in the attempt to establish whether a ground substance could be detected only in thick sections. We carefully examined and compared thin and thick sections of the same tissue in our own material and came to the conclusion that since no ground substance could be detected at any age in the thin sections, one may have the false impression of seeing an amorphous, lightly stained material between cells and cellular processes in the thicker sections from children and adults. We felt that this was due to the compactness of the neuropil, part of which, on a thick section, remained perforce out of focus. This provided a foggy background to the cell processes which were in focus at the moment, and led to a superposition of optical planes.

We became further convinced of this optical effect by examining the tissues under oil immersion ($\times$ 970) with very careful focusing. We could see in every field, and particularly well around cell bodies or in proximity to blood vessels, that whatever had appeared as an amorphous background showed in effect the fibrillar pattern of the neuropil after exact focusing with reduced intensity of light. We wish to emphasize that this effect was not so evident in the nervous tissue of embryos and fetuses in which the compactness of the neuropil is considerably less than in children and adults.

Furthermore, if the human central nervous tissue does possess a ground substance chemically related to the neutral mucopolysaccharides—as claimed by Hess to exist in the mouse,[1,3] rat[1] and guinea pig[2]—it should appear stained in red between the blue-stained neuropil with the Ritter and Oleson technique. With this staining procedure the neutral mucopolysaccharides appear bright red in color; but in our sections stained with this method, we could not detect any red-stained amorphous substance between the cell processes composing the neuropil. On the contrary, we observed that an optical effect identical to that noted in sections stained with the PAS-A and PAS-B reactions was demonstrable also in those stained with the Ritter and Oleson procedure, with the difference that in the latter the color of the background was light blue.

It is a well known fact that the central nervous system contains an intercellular fluid which exhibits such special characteristics as to appear somewhat different from the intercellular fluid of other tissues. The intercellular fluid cannot, however, be confused with a true

"ground substance" if we accept under such a designation only those substances which appear as more or less transformed intercellular sols, as in the case of the ground substance of other tissues, notably the various connective tissues.

It is conceivable that a ground substance is present in the walls of the blood vessels of the central nervous system. In these, a vague, light red stained, amorphous substance seemed to be present between the connective tissue fibers in the sections stained with the PAS procedures. In addition, a light blue tinge was visible in the same location when the sections were stained with the Ritter and Oleson method. In view of the results obtained with acetylation of the sections, whether or not treated with potassium hydroxide, we feel that it is rational to consider part of the vascular ground substance as similar in nature to neutral mucopolysaccharide, or at least possessing one to two glycol groups.

In addition, we think that since the blue stained material in the blood vessel walls was removed by testicular and pneumococcal hyaluronidase, acid mucopolysaccharides are present in this location also. We wish, however, to point out that although the existence in the central nervous system of a vascular ground substance composed of neutral and acid mucopolysaccharides is quite reasonable and acceptable, it is difficult to express a definite opinion in this regard because of the small size of the blood vessels and the difficulty in the evaluation of the histochemically stained preparations.

From this investigation we have gained the impression that the central nervous system of the human is devoid of a ground substance histochemically comparable to the ground substance of the other tissues, and that the only ground substance demonstrable in this location belongs to the vascular walls.

We cannot draw any conclusion from our morphologic data regarding the existence of the blood-brain barrier, a problem which is still quite controversial. Korting, Ostertag and Schmitz[19] have recently observed deposits of trypan blue in the brain of laboratory animals after injection of the dye preceded by subcutaneous injections of hyaluronidase. Kelentei and Foeldes[20] found streptomycin and penicillin in the cerebrospinal fluid of cats and rabbits following injections of the antibiotic agents either simultaneously with hyaluronidase or 15 minutes later. On the other hand, Ruckes and Diemer[21] could not detect any P^{32} in the brain of mice one hour after the introduction of the isotope, preceded by injection of hyaluronidase with or without histamine.

It seems that the data of Korting, Ostertag and Schmitz[19] and

Kelentei and Foeldes[20] are in agreement with our observations of the presence of a vascular ground substance in the central nervous system, its chemical nature and the digestibility of its acid polysaccharide components by hyaluronidase. Although we do not deny that a relationship might exist between this vascular ground substance and the blood-brain barrier, it is felt, nevertheless, that further investigation is necessary before a satisfactory solution of this problem is attained.

Summary

By means of histochemical methods (PAS-A, PAS-B, and Ritter and Oleson's stains) various segments of the central nervous system of man were examined in subjects ranging from 8 weeks of intrauterine life to adulthood.

No ground substance of the nervous tissue, histochemically comparable to the ground substance of other tissues, was observed in either the gray or the white matter. A ground substance was noted in blood vessel walls with structural features analogous to those encountered in the vessels of other organs. By histochemical techniques (acetylation, and digestion with testicular and pneumococcal hyaluronidase) this vascular ground substance was thought to be of acid and neutral mucopolysaccharide nature.

Although there was some indication that the vascular ground substance of the central nervous system might be related to the controversial blood-brain barrier, the authors feel that further related investigation is necessary.

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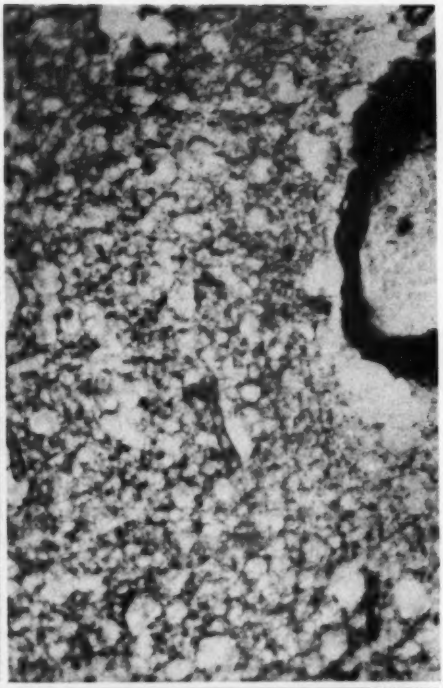
LEGENDS FOR FIGURES

FIG. 1. Cerebral cortex of human fetus, 17 weeks old. A small blood vessel is seen penetrating the outer layer from the leptomeninges. The meninges and the blood vessel walls show a strongly positive reaction with the periodic acid-Schiff stain which stains the nervous tissue lightly. PAS-A technique, section 12 μ thick. × 400.

FIG. 2. Cerebral cortex of human fetus, 28 weeks old. The neuropil is well formed but loose and lightly stained. No stained amorphous ground substance can be detected. PAS-A technique, section 12 μ thick. × 400.

FIG. 3. Cerebral cortex of human full-term newborn. The neuropil is still rather loose and stains very lightly as compared with the blood vessel fibers. No amorphous ground substance is visible in the nervous tissue. PAS-A technique, section 12 μ thick. × 400.

FIG. 4. Cerebral cortex of 3-year-old child. The neuropil is far more compact than in Figs. 2 and 3. No stained amorphous ground substance is visible in the nervous tissue. PAS-A technique, section 12 μ thick. × 400.



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IDIOPATHIC DEMYELINATING DISEASE IN YOUTH
A Study of Three Cases *

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The problem of idiopathic loss of myelin in large areas of the central nervous system, with accompanying changes in axis cylinders and glia, has defied solution despite the efforts of morphologists and experimental workers for more than 40 years. Schilder,[1] in 1912, first recognized juvenile demyelinating disease as a distinct entity and separated it from a miscellaneous grouping of infectious, degenerative, and neoplastic diseases. Favoring an infectious origin with primary effect upon the myelin, he suggested the term *encephalitis periaxialis diffusa*. A large number of publications relating to this disease have accumulated since Schilder's study; Gasul[2] reviewed 71 cases in 1930. These included heterogeneous disorders, such as those classified by Krabbe[3] as a "familial infantile form of diffuse brain sclerosis," occurring in young children and causing early death. In addition, those reported by Merzbacher[4] appeared to be transmitted predominantly by healthy mothers to male offspring who initially showed acute manifestations but subsequently followed a prolonged course. A third category, reported by Stewart, Greenfield and Blandy,[5] would probably be regarded by many as *neuromyelitis optica*. In 1946, Roizin, Helfand, and Moore,[6] in a survey of reported cases, tabulated some 20 different terms, many eponymic, which had been used by various authors to designate the disorders of myelin. Adams and Kubik[7] in 1952 reviewed the anatomic changes in many of the demyelinating diseases.

It is the purpose of this paper to present 3 cases of fatal demyelinating disease in young people with demonstration of certain histologic characteristics which suggest a close relationship to disseminated sclerosis.

Materials and Methods

The brains from the 3 patients were prepared in the following manner: Following brief preliminary fixation in 4 per cent formalin, and transection through the midbrain, the brain was coronally sectioned at intervals of 1.0 cm. After further formalin fixation, selected

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slices were embedded in celloidin and serially sectioned and stained by the following techniques: hematoxylin and eosin, Nissl's crystal violet stain for cellular detail, the Weil modification of the Weigert-Pal technique for myelin, Mallory's phosphotungstic acid hematoxylin stain for glial elements, and the Bodian silver method for axis cylinders. Additional smaller blocks were selected and embedded, usually in paraffin, but occasionally in celloidin. For these smaller blocks, the following stains or staining methods were used in addition to those mentioned above: the original Weigert method for myelin, the Holzer stain for glial fibers, Davenport's alcoholic silver nitrate method for axis cylinders, Mayer's mucicarmine stain for mucin, Wilder's reticulum stain, and the toluidine blue stain for metachromasia. Frozen sections were prepared and stained with Sudan IV and Sudan black B for neutral fat, with Cajal's gold-sublimate method for astrocytes, and Schultz's sterol reaction method for cholesterol.

Report of Cases

Case 1

Clinical Summary. A 16-year-old Negro boy was admitted to the Cleveland Clinic Hospital on October 4, 1951. He had been in excellent health until 3 weeks previously when he had a "cold" with headache and "stopped" nose for 5 days. One week before entry, he noted numbness of his toes and feet, which gradually ascended to the knees and lower thighs. Two days before admission, he began to have progressive difficulty in walking, and for 16 hours prior to admission he could no longer walk without aid. He had no rectal sensation, had been constipated for 3 days, and had voided only once during the 24 hours before admission.

Physical examination revealed a well-nourished boy with normal temperature and a regular pulse of 84. A grade I apical systolic murmur was present. There were hypesthesia below D10, diplegia of the lower limbs, hyperactive tendon reflexes in the legs, unsustained ankle clonus on the left, bilaterally positive Babinski reflexes, absent vibratory and position senses, and absent abdominal reflexes. The upper extremities were not abnormal, the cranial nerves were intact, and an ophthalmoscopic examination was normal.

Laboratory examination revealed: hemoglobin 12.2 gm. per 100 ml., white blood cell count 9,900 per $mm.^3$. A serologic test for syphilis was negative; blood sugar, 77 mg. per 100 ml.; and urinalysis normal. Lumbar puncture demonstrated a clear colorless fluid under pressure of 19 cm. of water and a normal Queckenstedt's sign; 15 ml. of fluid were removed with a final pressure of 15 cm. of water. Cerebrospinal fluid contained 300 red blood cells and 8 white blood cells per $mm.^3$ (85 per cent lymphocytes); protein content was 40 mg. per 100 ml. Roentgenograms of the chest and thoracic spine were normal.

Four days after admission, the patient complained of a "dead feeling" in the right hand and was noted to be lethargic. Paralysis of the lower extremities was complete, and all reflexes were absent. Combined cisternal and lumbar puncture showed no evidence of subarachnoid block. On the next day, definite weakness in the right arm, nystagmus, and diplopia were present. Eight days after admission lethargy was still evident, and the patient complained of blurring of vision. Cortisone (25 mg. 3 times daily) and ACTH (5 mg. 2 times daily) were administered.

Thirteen days after admission, the patient responded only to painful stimuli, and the temperature was 100° F. Cortisone therapy was discontinued.

On the 16th day, paralysis of the internal rectus muscle of the left eye developed. A positive right Hoffmann's sign was elicited. The administration of ACTH was discontinued. On the 18th hospital day the patient became semicomatose; the Babinski reflexes were positive, bilaterally; reflexes in the upper extremities were depressed. The lungs filled with moist rales; the temperature rose to 104° F. He lapsed into deep coma, and died 24 days after admission.

Pathologic Features

The brain weighed 1,300 gm. The dura and the leptomeninges were normal and without focal thickening. The cerebral substance appeared somewhat swollen with flattening of the gyri and sulcal narrowing. The basilar structures of the brain were unchanged; the cerebral arteries were normally distributed. Coronal sections revealed good demarcation between the gray and the white matter throughout the brain. The frontal portion of the right *centrum semiovale* contained a faintly gray, well-demarcated, slightly depressed central plaque, measuring 1.5 by 0.8 cm. The cut surfaces of the remaining sections showed no gross abnormalities.

Multiple transections of the spinal cord revealed a solitary, gray, translucent, softened area which appeared to occupy the entire diameter of the upper thoracic spinal cord and extended down the cord for a distance of 1.5 cm.

Histologic examination of the right frontal and right occipital lobes disclosed several foci with an unusual type of demyelination. These were best observed by gross inspection of the celloidin total mounts stained by the Weil modification (Fig. 1). Concentric rings with varying degrees of loss of staining were present. These rings differed from those described by Baló,[8] in that there were no intervening areas of dark-staining, intact, myelin sheaths. For purposes of description, the term "target" demyelination will be used. Centrally the target lesions showed pronounced loss of nerve fibers. However, the axis cylinders were slightly better preserved than the myelin sheaths. Surrounding the central area in each focus was a darker, faint ring of fragmented myelin sheaths; surrounding this ring was another zone of intense demyelination; and finally, at the rim of the target lesion, there was a faint area of demyelination with sharp borders (Fig. 2). The severely demyelinated regions were infiltrated by many macrophages, containing large amounts of sudanophilic material. Many of the remaining swollen myelin sheaths were irregularly sudanophilic. Examination of these areas with the polarizing microscope revealed numerous crystals free in the white matter and engulfed by macro-

phages. Occasionally, with polarized illumination, the crystals appeared red in Sudan IV preparations. They were soluble in lipid solvents such as ether and xylol.

The occipital lobes contained large areas of paraventricular demyelination that extended throughout almost the entire expanse of the white matter at the postero-lateral extremities of the lateral ventricles. Superiorly, the demyelination involved only a third of the occipital lobe. Some of these foci also showed the peculiar laminations of destruction seen in the "target" lesions. Other broad areas of demyelination extended anteriorly into the parietal lobes in the paraventricular regions; the sagittal striae were especially affected. In addition, numerous, small (up to 4.0 mm.) almost punctate foci with loss of myelin, fragmentation of axis cylinders and infiltration by macrophages, were present. These lesions rarely involved the arcuate fibers or extended into the cortex. It must be noted that in many portions of the cerebral white matter, plaques appeared in approximation leaving only faint separating rims of degenerating myelin. It was possible to decolorize this degenerating myelin easily during staining with the Weigert method, and several small plaques were thus transformed into one large confluent lesion.

In all of the degenerating areas, examination of frozen sections with polarized light revealed the same soluble crystals limited to the areas of demyelination and to the accumulations of lipoid within macrophages (Figs. 3 and 4). The axis cylinders here showed varying degrees of degeneration (Fig. 5). In some foci they had almost completely disappeared; in other areas, some were intact but others showed irregular swelling, fragmentation, end-bulb formation, and tortuosity of the filaments. The astrocytic alteration within the lesions was extreme; Cajal's stains revealed the astrocytes to be greatly swollen, with thickening and, in some, fragmentation of processes (Fig. 6). These elements appeared to be increased only in size and not in number. They often were multinucleated but did not contain mitotic figures.

In addition to the involvement of the hemispheres, a large plaque with a serpiginous, sharply marginated border, and several smaller foci of demyelination were present within the pons. A small oval lesion lay adjacent to the right dentate nucleus of the cerebellum. In the upper thoracic cord, another extensive area of demyelination was evident. This spared little of the long fiber tracts and displayed the same intense reaction characterized by a loss of myelin-staining qualities with Weigert's method, and by diffuse lipophage activity with

perivascular accumulations and deposition of crystals. There was no evidence of tract degeneration except in the local lesion.

Case 2

Clinical Summary. A 10-year-old white boy was first admitted to the Cleveland Clinic Hospital in April, 1950. Throughout his entire life he had had frequent headaches and motion sickness. During the year prior to entry, the headaches had become more severe and had been associated with nausea, vomiting, and vertigo with a tendency to fall to the right. Approximately 4 weeks before admission, the patient was awakened one morning by a severe generalized headache accompanied by vertigo, nausea, and numbness in the right hand, foot, and leg. The sensory change was associated with weakness on the right side, including drooping of the right corner of the mouth and diplopia. The symptoms persisted for 10 days and then gradually subsided but recurred after several days.

Physical examination demonstrated rotary nystagmus, coarser to the right. There were diminished right corneal reflex and extreme dysmetria, asynergy, and intention tremors of the right arm and leg. Spinal fluid examination revealed a clear fluid with some xanthochromia, normal pressure, and normal dynamics. The cerebrospinal fluid contained 8 cells per $mm.^3$ and 19 mg. of protein per 100 ml.; the serologic test for syphilis was negative. An electroencephalogram was subnormal with generalized slow waves, more pronounced on the left posteriorly. Visual fields, on funduscopic examination, were within normal limits.

A tentative diagnosis of right cerebellar tumor was made, but a pneumoencephalogram was normal, and arteriograms revealed no abnormalities. Following these procedures, the child recovered and was discharged 8 days after admission.

When he was seen again 2 months later, he appeared in good health with no complaints. No nystagmus was noted. The right Achilles reflex still was hyperactive, but all other tendon reflexes were normal. Rossolimo's reflex was present on the right foot. Cerebellar signs were within normal limits. He remained well for 4 months and then one morning awakened with frontal headache, followed by numbness and weakness of the right arm and leg. Within 2 days the symptoms subsided spontaneously. During the next year he had 3 more similar episodes. In October 1952, the child developed an upper respiratory infection, following which he complained of headache and dizziness, and was unable to walk in a straight line. He vomited several times, became incoherent, and confused. Five days later he was readmitted.

Neurologic examination revealed right facial drooping, positive Babinski signs bilaterally with the right greater than the left, a right hemiparesis, and no nystagmus. Lumbar puncture showed normal dynamics. The cerebrospinal fluid contained no cells; the protein level was 25 mg. per 100 ml. An electroencephalogram revealed a striking disparity between the two hemispheres with pronounced delta activity over the left side, particularly the parietal, temporal and occipital lobes, but little or no delta activity over the right cerebral hemisphere.

The next day the patient suffered a severe episode of *status epilepticus*, followed by what appeared to be a state of decerebrate rigidity with convulsions. Lumbar puncture showed normal pressure and normal dynamics. He remained comatose for one week with repeated convulsions, and then died with extreme rigidity.

Pathologic Features

The brain weighed 1,380 gm. The meninges and its reflections were normal. The external cerebral surfaces had normal convolutions, and

the basilar structures of the brain showed no external deformities. The cerebral vasculature was normal. Multiple coronal sections revealed numerous, irregularly distributed, variably sized, translucent, gray areas within the white matter. The largest area, 1.0 cm. in diameter, was in the midportion of the superior frontal gyrus. Several smaller foci were encountered in the region of the motor area on the right and discrete lesions were indistinctly visible in the immediate vicinity of the posterior horns of the ventricles. A small (1.0 by 0.4 cm.), depressed, gray plaque was seen in the region of the decussation of the *brachium conjunctivum*. Gross findings in the remaining brain stem and cerebellar sections were normal.

On histologic examination, staining with Weigert's method revealed that the occipital lobes were almost completely demyelinated (Fig. 7). However, the demyelination was of peculiar nature in that even where the lesions seemed to be broad and extensive, a curious mottling was present. The lesions exhibited a marginal mosaic pattern and appeared to be subdivided by small black strands. In addition to the extensive lesions, small adjacent foci with loss of staining could be seen. Study of the finer histologic detail showed the mottling to be due to various stages of degeneration. Within the large lesions, isolated foci of degenerating myelin could still be identified, while in other areas, no myelin could be demonstrated at all. In the latter regions, a severe infiltration by gemistocytic astrocytes and macrophages was present. The axis cylinders were often greatly degenerated and at times had disappeared entirely (Fig. 8). Astrocytes were swollen, with thickening and fragmentation of their processes. The marginal mosaic pattern was caused by groups of degenerating nerves, which, although retaining an affinity for myelin-sheath stains, were swollen and fragmented (Fig. 9). The neighboring smaller lesions were similar. This peculiar histologic picture was believed to result in part from the fusion of numerous plaques within the occipital lobes. It was possible, by successive decolorations of the sections which had been stained by Weigert's method, to remove all intervening boundaries completely and to convert the entire process into one vast area of apparent demyelination. The arcuate fibers were spared throughout both occipital lobes.

The parietal lobes showed alterations resembling those in the occipital lobes. The white matter was the seat of extensive involvement, with a mosaic pattern due to contiguous plaque formation (Fig. 10). Occasionally, smaller foci exhibited only pallor and early beading of the myelin without a cellular reaction. Rarely, these

small lesions had a perivascular distribution. Fat stains on frozen sections of parietal and occipital lobes showed lipid within macrophages and a tendency of these cells to aggregate perivascularly. Crystals similar in appearance and distribution to those observed in case 1, were present (Figs. 11 and 12). No crystals were identified in uninvolved areas. The sections of the left anterior frontal lobe contained a large area of demyelination, again with marginally contiguous plaques but little cellular reaction. Small foci of demyelination affecting the arcuate fibers were present in both the frontal and the parietal lobes. These, however, did not extend into the overlying cerebral cortex. The cortical sections, without exception, contained intact neurones. The temporal lobes appeared to be normal.

Sections of the midbrain showed a large plaque with irregular but sharp margins, which contained many macrophages with sudanophilic content. In this region, vessels were rimmed with crystal- and lipid-laden cells at rather great distances from the area of myelin breakdown (Figs. 13 and 14). The pons and cerebellum also contained small discrete foci of demyelination. The sections stained with toluidine blue demonstrated no metachromatic material, and mucin stains were unrevealing. The optic nerves and tracts were normal. Only the upper cervical cord was sectioned and the findings here were normal; no tract degeneration was apparent.

*Case 3**

Clinical Summary. A 15-year-old white boy was first examined at the Cleveland Clinic on May 25, 1951. He was an only child and had been born at term, by normal delivery. The child's early development was normal; he talked at the age of one year and had a good vocabulary by 15 months. He walked at the age of 14 months, but was of small stature. He appeared to do well in kindergarten, and not until he started in grade school was it noted that he was somewhat deaf. Subsequently, he did not do well in school, and except for single words, never learned to read. He could do simple arithmetic and had a retentive memory for people and places.

During the 5 years prior to admission, the patient showed little growth, was very thin, and was bothered by a coarse tremor of the hands and head. However, he learned to use power tools capably. The only previous illnesses noted were measles at the age of 8 months and scarlet fever at the age of 12 years. His tonsils had been removed. His father had had syphilis prior to marriage; this had been suitably treated. Serologic tests for syphilis of the mother had always been negative.

Physical examination revealed a small thin boy, 54¼ inches in height and 50 pounds in weight. There were bilateral pes cavus of the feet, and a lack of bodily hair and of development of external genitalia.

The pupils proved to be equal and normally reactive to light and accommoda-

* Dr. Lester Adelson of the Cuyahoga County Coroner's Office provided the necropsy protocol and the brain tissue for examination.

tion. There was some limitation of extraocular eye movements, especially in the vertical axis. The upper extremity reflexes were slightly diminished, but the Holmes rebound sign was positive bilaterally. The abdominal reflexes and cremasteric reflexes were intact. The patellar reflexes were hyperactive bilaterally. Achilles tendon reflex was absent on the right due to contracture of the tendon; it was greatly exaggerated on the left and was accompanied by slight clonus. The Babinski and the Rossolimo signs were positive on the right and faintly positive on the left. The patient walked on a broad base with both ataxic and spastic gait. Asynergy of hand and arm movements was noted. He had a slow, almost explosive type of speech. The optic discs were flat and pale.

Laboratory examination revealed a hematocrit reading of 43 cc. per 100 ml.; white blood cell count of 6,350 per $mm.^3$; serologic tests for syphilis were negative; blood sugar, 85 mg. per 100 ml. The urinalysis showed a specific gravity of 1.030 with no microscopic abnormalities. Spinal fluid examination revealed a slightly xanthochromic fluid, positive reaction to the Pandy test, and 8 lymphocytes per $mm.^3$ The protein content of the spinal fluid was 98 mg. per 100 ml. A serologic test for syphilis was negative. The colloidal gold curve was flat (0000000000). An electroencephalogram revealed a generalized abnormally slow pattern with high voltages. More slow waves were noted in the right parietal and motor regions than on the left side.

A tentative diagnosis of Merzbacher-Pelizaeus disease (*aplasia axialis extracorticalis congenita*) was made. The mother refused to permit the child to undergo further studies. The patient returned home where he died suddenly two months later, apparently during a convulsive episode.

Pathologic Features

The brain weighed 1,000 gm. The meninges and their reflections were not remarkable. There were no external malformations of the cerebral convolutions and no evidences of brain atrophy. Multiple coronal sections through the cerebrum revealed the white matter to be greatly altered. Irregular areas consisting of homogeneous, translucent, gray tissue were scattered throughout. The change was most pronounced in the upper portion of the *centrum ovale*, extending to varying degrees into the frontal, parietal and occipital lobes. The temporal lobe seemed to be free of alteration. The process, while not completely symmetrical, was bilateral. However, even in the areas where the degree of involvement appeared most pronounced, the arcuate fibers just beneath the gray matter of the convolutions, were spared. The deeper portions of the translucent lesions were blended imperceptibly into the white matter. The basal ganglia and internal capsule were well preserved. Step sections through the midbrain, pons, medulla, and cerebellum revealed no gross abnormalities; there was good demarcation between gray and white matter. The cerebral vessels were distributed in a normal manner and were thin and pliable. Multiple sections throughout the entire spinal cord exhibited no gross abnormalities.

The histologic lesions in this case differed distinctly from those in

cases 1 and 2. The observations on examination of coronal sections of the hemispheres stained for myelin corresponded well with the gross anatomic features. There were extensive, similar, but not symmetrical areas of demyelination present throughout both cerebral hemispheres. The arcuate fibers were intact, but the white matter of the convolutions was almost completely destroyed. In the *centrum ovale,* the devastated areas blended irregularly with what appeared to be normal myelinated fibers (Fig. 15). The occipital lobes were diffusely affected, except for the fibers in the paraventricular region, calcarine fissure, and arcuate areas (Fig. 16). The demyelinated areas were spongy in appearance with a few intact myelin sheaths and axis cylinders coursing through the destroyed tissue. No cellular reaction or metachromasia were noted. Oligodendroglia were present, but there were no giant astrocytes or evidences of gliosis. Within the borders of the lesion, swollen axis cylinders or beaded myelin sheaths were occasionally seen, but for the most part the process appeared inactive. No small lesions were noted in relation to the larger foci, nor was there evidence of damage within the midbrain, pons, or brain stem. Demyelinated areas, however, were found in the central white matter of the cerebellum. Sections of the spinal cord revealed the long tracts to be intact.

Comment

Certain features of cases 1 and 2 require further emphasis. The presence of numerous crystals within the degenerating lesions was especially intriguing because only one other mention of this occurrence was found in the literature—a report by Greenfield and King[9] on disseminated sclerosis. Since the crystals were soluble in the lipid solvents, they may constitute a type of free fatty acid. A study of frozen sections from 2 cases of *neuromyelitis optica* and 2 cases of disseminated sclerosis revealed similar soluble crystalline material. In contrast, crystals have not been demonstrated in the borders of cerebral infarcts or in the degenerating myelin surrounding neoplasms of either primary or secondary nature. Moreover, crystals were not found in the lesions of a case of progressive metachromatic leukoencephalopathy,* or in 2 cases of acute hemorrhagic leukoencephalopathy.

It would appear from these observations that a specific mechanism of myelin destruction is present in disseminated sclerosis, *neuromyelitis optica,* and in the first two cases cited in this report. Cases 1 and 2 have other features in common with disseminated sclerosis.

* Tissues were furnished by William Sinclair, M.D., of Cleveland, Ohio.

The focal lesions cannot be distinguished from those of the latter disorder or from those described in many cases of diffuse sclerosis (Schilder's disease). By greater decoloration during staining by the Weigert method, the lesions could be made to resemble those of diffuse sclerosis. This suggests that at least a portion of the large areas of demyelination originated by fusion of contiguous plaques. Roizin, Helfand, and Moore[6] considered this phenomenon as "transitional demyelination." The multiple lesions suggest an underlying process very similar to that seen in multiple sclerosis. One may only speculate that the factor of age may conceivably have been a modifying feature in case 2.

The factors concerned with the variable degrees of glial stimulation are unknown. Although the character of the glial proliferation has been used as a basis for differentiation of cases of diffuse leukoencephalopathy with sclerosis from those without sclerosis,[10,11] such separation does not appear entirely justified. Conceivably, astrocytes may be absent locally in areas of destruction in some cases so that no subsequent proliferation is possible. On the other hand, in others some may survive and give rise to the development of gliosis.

The alterations encountered in the third case may represent the end point of the lesions evident in the first 2 cases. The process had apparently run its course completely and examination showed only residua of the degeneration which at one time had probably been acute. There was no evidence in any of the 3 cases that a degeneration of oligodendroglia or other glial elements had played any role in the production of the lesions. Greenfield's[12] studies of the association of progressive cerebral sclerosis and primary degeneration of interfascicular glia are intriguing, but that association was not present in the cases presented here. In the relatively normal areas, interfascicular glia showed a normal distribution.

In none of the 3 cases was there evidence of familial disorder, nor was metachromatic material observed in the altered tissues. In 2 of the 3 cases, a focal pallor of the stained myelin of the outer zone surrounding the target lesion, and a similar abnormality in other areas, often of large size, was observed in advance of any cellular proliferation. This peculiar circumstance offers some substantiation of the hypothesis that there exists in such cases a myelinolytic ferment which may diffuse slowly into the white matter. Autolyzed brain apparently is antigenic, as the studies of Schwenker and Rivers[13] and others have shown. It is possible that in some persons the myelin reacts abnormally to noxious stimuli which are ordinarily considered to be trivial. Evidence is accumulating slowly to support this hy-

pothesis. The rare cases of acute demyelinating encephalomyelitis following respiratory disease[14] are possible examples of such a mechanism.

The "allergic theory"[15] may have significance in the pathogenesis of the disorder in case 1. It appeared that demyelination had progressed to a certain point, and then a succeeding wave, possibly related to sensitization, affected contiguous areas of the myelin with resultant breakdown. The histologic pattern is consistent with the effects of repeated insults within the same general region.

Two of the 3 cases appeared to have some association with simple upper respiratory infection. In one (case 1) it shortly preceded the onset of the disease, and in the other (case 2) it was the initial feature of the final episode that led to death.

Summary

Three cases of an idiopathic loss of myelin in the central nervous system of young boys have been presented. A wide variation in the clinicopathologic features is represented. In the first case, the changes were acute and progressive without previous history; in the second case, multiple exacerbations of the disorder had occurred; and in the third, no definite time of onset could be ascertained.

The lesions in the first two had many alterations in common. The most striking of these changes was the presence of crystalline lipid products in association with the more recent breakdown of myelin. These crystals were also found in lesions of disseminated sclerosis and other closely related entities, such as *neuromyelitis optica*. The lesions in the third case were old and for the most part quiescent. No crystalline material was demonstrated. The possibility that the presence of a crystalline material represented a different pathway for the breakdown of myelin in disseminated sclerosis and related lesions has been considered.

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LEGENDS FOR FIGURES

FIG. 1. Case 1. Celloidin total mount showing "target" demyelination and other small early areas of myelin loss. Weigert's stain.

FIG. 2. Case 1. Concentric rings of demyelination of varying stages; the most extensive is upper right. Weigert's stain. × 25.

FIG. 3. Case 1. Acutely damaged zone in thoracic spinal cord, showing extensive macrophage activity with perivascular aggregation. Frozen section stained with Sudan IV. × 85.

FIG. 4. Case 1. Polarized light photograph of Figure 3, showing distribution of small birefringent crystals. × 85.

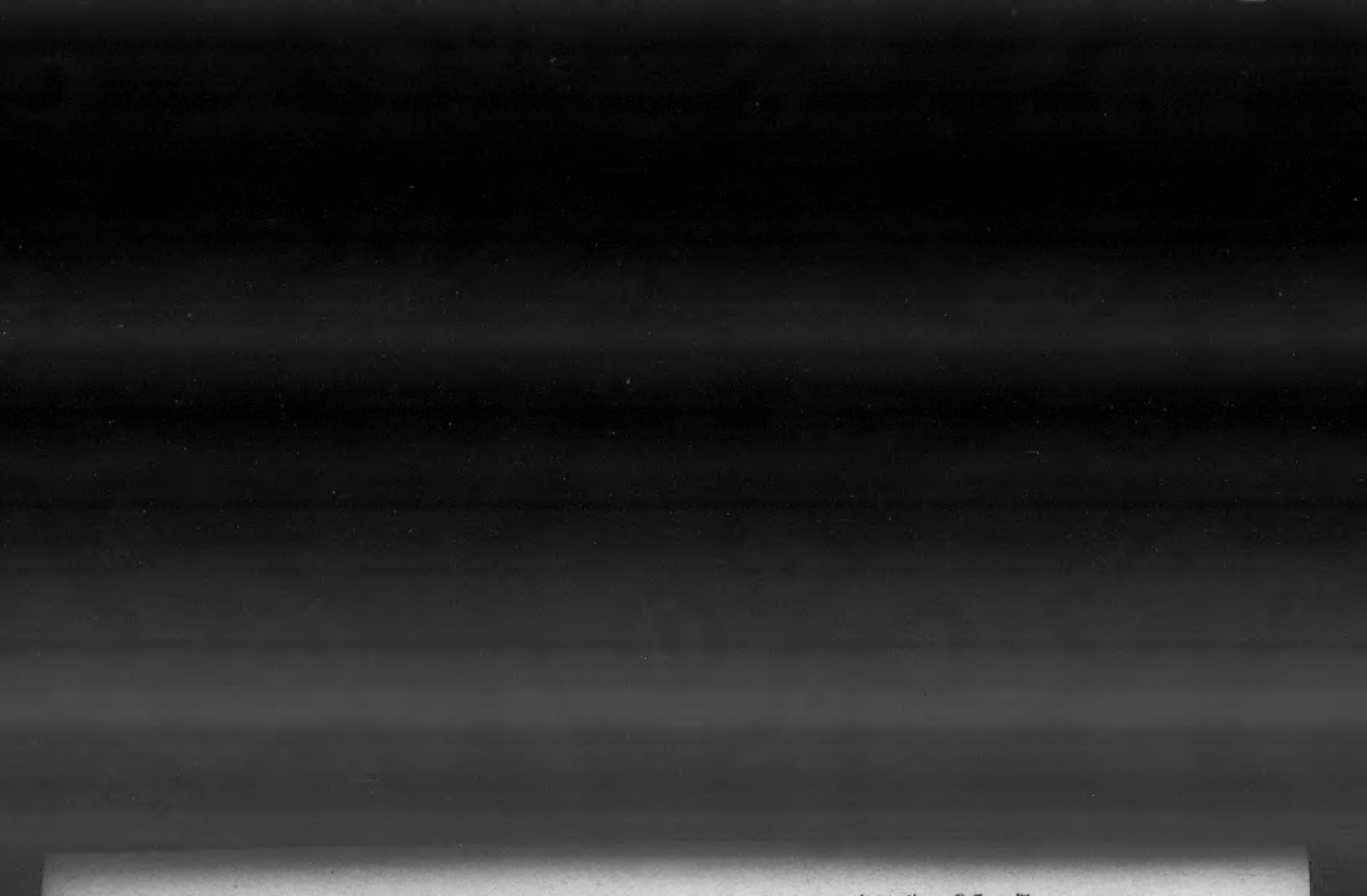

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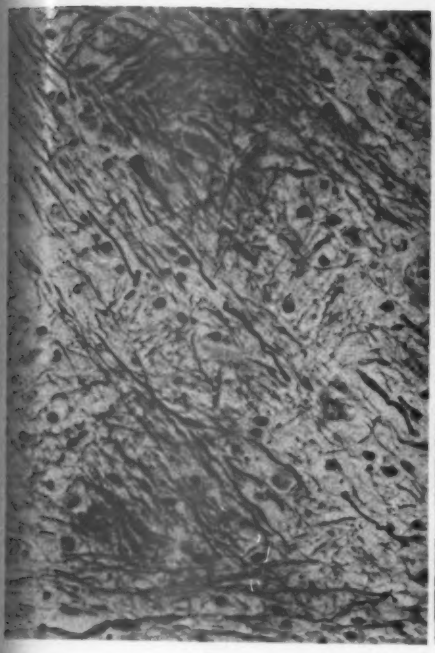
FIG. 5. Case 1. Axis cylinder degeneration in focus of demyelination. Bodian's stain. × 270.

FIG. 6. Case 1. *Top.* Uninvolved area containing normal astrocytes. *Bottom.* Astrocytic swelling with fragmentation of cell processes in affected area. Cajal's stain. × 270.

FIG. 7. Case 2. Total mount of left occipital lobe showing extensive demyelination in part resulting from fusion of plaques. Weigert's stain.

FIG. 8. Case 2. Extensive axis cylinder degeneration. Bodian's stain. × 260.



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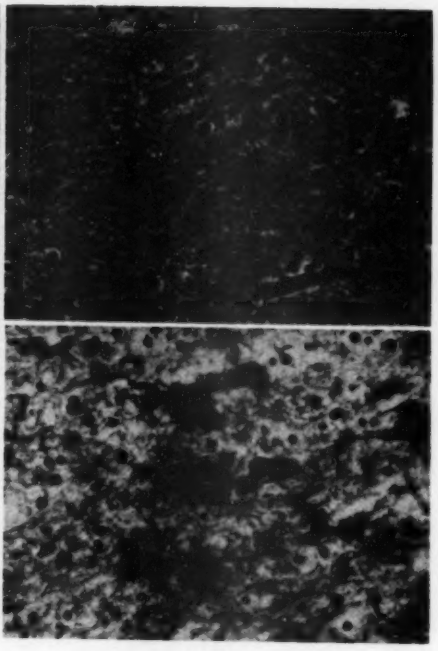

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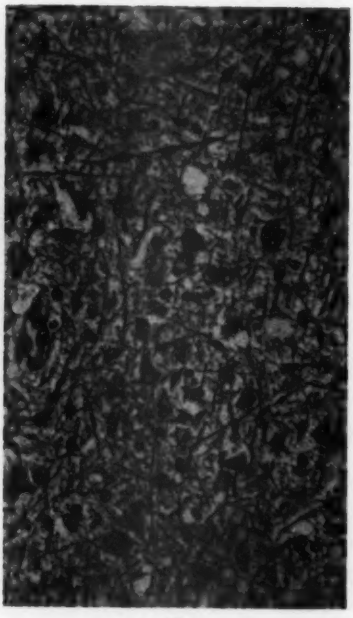

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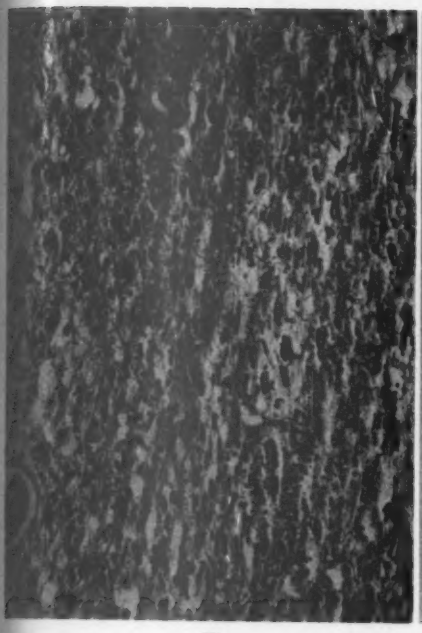
FIG. 9. Case 2. Myelin degeneration in linear zone between two areas of older degeneration. Weigert's stain. × 130.

FIG. 10. Case 2. Coronal section of left cerebral hemisphere at level of parietal lobe. The mosaic pattern is still faintly visible. Weigert's stain. Celloidin total mount.

FIG. 11. Case 2. Fat stain, showing accumulations of sudanophilic material in macrophages with perivascular concentration. Frozen section stained with Sudan IV. × 80.

FIG. 12. Case 2. Polarization of same area as seen in Figure 11, showing crystalline material. Polarized light. × 80.



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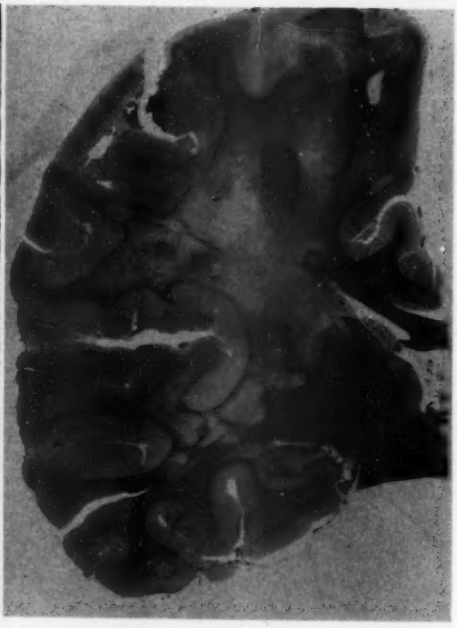

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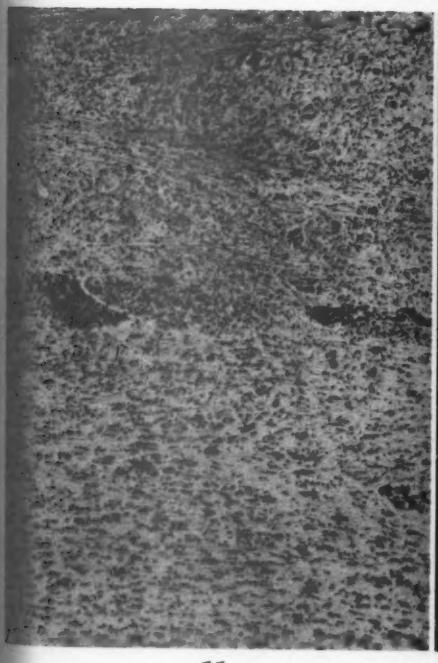

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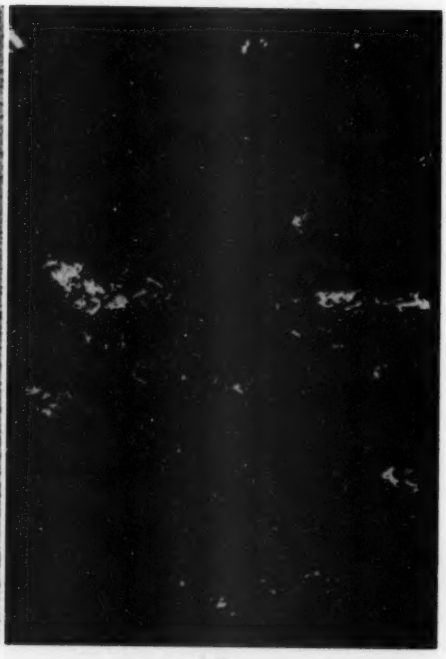

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FIG. 13. Case 2. Small veins in midbrain partially ringed by macrophages. Lesion is not in vicinity of active demyelination. Frozen section stained with Sudan black B. × 80.

FIG. 14. Case 2. Polarization of same area as seen in Figure 13, showing crystals present in same areas as the sudanophilic material. Polarized light. × 80.

FIG. 15. Case 3. Patchy extensive loss of myelin in coronal section of left cerebral hemisphere, frontal lobe. Celloidin total mount. Weigert's stain.

FIG. 16. Case 3. Coronal section of right occipital lobe with the same extensive myelin loss. Note sparing of visual and paraventricular regions. Celloidin total mount. Weigert's stain.

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